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The Effects of Sodium Chloride, Sodium Sulfate, and Consolidated Tailings Water on Jack Pine (*Pinus banksiana* Lamb.) Seedlings

by

Jennifer A. Franklin

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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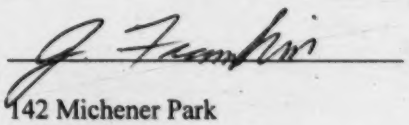

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The Effects of Sodium Chloride,. Sodium Sulfate, and Consolidated Tailings Water on Jack Pine (*Pinus banksiana* Lamb.) Seedlings" in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Forest Biology and Management.

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Abstract

The Athabasca Oil Sands deposit in Northern Alberta is the site of large-scale mining operations (Syncrude Canada Ltd. and Suncor Energy Inc.) that produce relatively saline tailings water, sand and fine tailings. Reclamation goals for the site of current mining operations include the establishment of a productive forest, however the salt tolerance of many native forest plants, including jack pine, is largely unknown. The objective of thesis research program was to determine the effects of water associated with composite tailings (CT water) and its main salts, NaCl and Na_2SO_4 on the growth and physiology of jack pine (*Pinus banksiana* Lamb.), and to investigate the mechanisms of salt injury and tolerance.

Jack pine seedlings were grown in sand or solution culture, and exposed to solutions of NaCl, Na_2SO_4 or CT water in a series of experiments. Treatment of one-year-old seedlings with NaCl had a more detrimental effect than did isomolar Na_2SO_4 with respect to most of the parameters measured, suggesting that ion toxicity is the dominant effect. While Na_2SO_4-treated plants showed reduced tissue K^+ and Ca^{2+}, of which deficiencies are often associated with salt stress, plants treated with NaCl had significantly increased levels of several nutritional elements. Shoot Na was greater in NaCl-treated plants than in plants treated with Na_2SO_4 at equivalent Na levels, and was correlated with tissue injury only in the former. Tissue electrolyte leakage was more closely related to treatment Cl level, than to Na level, suggesting that Cl is associated with increased membrane permeability. Transpiration rates were similar in all salt treatments, and the greater translocation of Na in NaCl-treated plants was the result of greater root permeability to Na.

Seedlings treated with CT water exhibited similar growth inhibition and injury to plants treated with salts, and injury was related to both shoot Na and Cl levels. The uptake of mineral nutrients may be influenced by the high pH of the treatment solution. Because jack pine appears to be sensitive to substrate Cl, planting of this species is recommended only on sites where Cl is low.

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Chapter I

Introduction

Salinity is common in soils throughout the world, and is one of the leading factors limiting the growth of agricultural crops. Saline soils develop where groundwater flow is shallow, and evaporation concentrates salts near the soil surface. In Alberta, naturally saline soils cover approximately 14,800 hectares, mainly in depression bottoms and along coulee slopes (Alberta Agriculture, 1995). Sodium sulfate and Mg_2 SO_4 are the most common salts found in Alberta, although chloride salts occur where rocks of marine origin are present. In central and southern Alberta, agricultural land may become salinized by the evaporation of irrigation water. Regional salinization may also occur from spills of brine, used in the oil industry, and from salts brought to the surface by mining activity.

The Athabasca oil sands reserves north of Fort McMurray, Alberta, contain an estimated 625 billion barrels of heavy crude oil (bitumen). The reserves are contained in the McMurray formation, which consists of Cretaceous age fluvial, estuarine, and marine sand deposits that lie over Devonian rocks, and are overlain by sandstone and shale (Jardine, 1974). The ore is recovered by surface mining, and hot water is used to extract the bitumen which is then upgraded to synthetic crude oil. These reserves are being developed aggressively, with two commercial mining operations (Syncrude Canada Ltd. and Suncor Energy Inc.) currently in production and several other companies in pre-production stages. Large volumes of solid and aqueous tailings remain after the separation of bitumen from the oil sand ore, and this will necessitate the eventual reclamation of land areas in excess of 50,000 ha. Current tailings management practices involve the containment of the tailings within settling basins, as well as a technology that entails the re-mixing of the fine and coarse components along with gypsum as a chemical coagulant to produce a non-segregating material known as consolidated or composite tailings (CT) (Matthews et al, 2000). This mixture is deposited as slurry from which solids-free water is released. The goal is to produce a deposit that becomes trafficable, allowing equipment movement, and reclaimable within a shorter period than without this

CT treatment. A large volume of water will remain in this material and is expected to be expressed over a number of years as the deposit settles further. These tailings and associated pore waters (CT water) are more saline than the original soils, with Na and Cl being derived primarily from the ore, while most of the SO_4 comes from the gypsum treatment (MacKinnon et al., 2000). Conductivity of contained water is expected to exceed 4dS/m in some reclamation materials, and may inhibit the growth of some plant species. Other characteristics of CT water that could potentially affect plant growth include elevated levels of boron, fluoride, naphthenates, and a relatively high pH (Renault et al., 2000).

Jack pine (*Pinus banksiana* Lamb.) is native to the Canadian boreal forest, with a range extending from Nova Scotia to northern British Columbia. This early-successional species is typically found on nutrient-poor sandy soils (Cayford et al., 1967), and may therefore be a suitable reclamation species for these sites. Because jack pine is a dominant tree of mesic to xeric sites in the pre-disturbance ecosystem, and has potential commercial value, the re-establishment of this species is highly desirable. Once established, reclamation sites may appear similar to natural stands, as these tend to be even-aged and low in diversity. However, little is known of the tolerance of jack pine to salinity.

This research is a part of a larger project that began in 1995, investigating the effects of tailing materials on plant growth and physiology. These have included field and greenhouse studies, utilizing a number of plant species most of which are woody species native to the Fort McMurray area. Previous studies on conifer species have found that while lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.), white spruce (*Picea glauca* (Moench) Voss) and black spruce (*Picea mariana* (Mill.) Britton et al.) are only moderately tolerant of CT water, they exhibit a large amount of individual variation in response (Renault et al. 1998). Results of previous research within this project also suggest that salinity in oil sands tailings materials may be the factor having the greatest negative effect on plant growth. The predominant ions in CT are Na^+, Cl^-, and SO_4^{2-}, with the anions occurring in various proportions.

The main objectives of the research reported here are to characterize the effects of CT water and its principle components, NaCl and Na_2SO_4, on jack pine growth and

physiology, to investigate the mechanisms by which these salts and CT water result in injury to jack pine seedlings, and to examine possible tolerance mechanisms in this species. The determination of components in CT water that are injurious to jack pine, and the mechanisms of injury, will help reclamation planners to select tailings management options that are acceptable for jack pine reforestation and to select sites suitable for planting jack pine.

The apparent high genetic variability in the response of conifers suggested that selection of more salt-tolerant individuals could be possible, and this hypothesis was tested under field and controlled-environment conditions (Chapter III). The main objective of this study was to produce seedlings that would have a greater survival rate, when planted on reclamation sites impacted by CT materials, as a result of pre-treatment with salt. Growth-room experiments were conducted to test the hypothesis that Na, Cl, and SO_4 are the predominant factors in CT water affecting jack pine growth (Chapter IV). Shoot concentrations of salt ions, macronutrients and micronutrients in control and CT-treated plants were analyzed, and the relationship of these factors to growth and injury was examined. Because very little was known about the response of jack pine to CT water, the effects of CT water on jack pine growth and injury were characterized. The relative alkalinity of CT water may have a negative impact on plant nutrient status, therefore, a further objective of this study was to determine the effects of CT water on nutrient uptake.

Due to differences in the ore and in processing, the relative proportions of Cl and SO_4 will vary between CT deposits. It is therefore necessary to understand how the effects of these two salts differ. The effects of NaCl and Na_2SO_4 on jack pine seedling growth and injury were compared. This study was designed to test the hypothesis that growth and injury are related to shoot concentrations of salts and nutritional elements (Chapter V). The potential mechanisms of salt injury were investigated in a study that tested the hypothesis that Cl is responsible for primary injury to cell membranes (Chapter VI). The second objective of this study was to understand how salt ions and nutritonal elements move and accumulate within the plant as a result of NaCl and Na_2SO_4 salinity. The characterization of salt accumulation in different tissues within the plant provides

insight into potential mechanisms of salt tolerance in this species, and may be helpful in predicting the long-term effects of salt.

Ion translocation from the root to the shoot may differ in plants treated with different salts, and the hypothesis that these differences in transport and shoot accumulation are the result of differences in transpiration rate was tested (Chapter VII). The objective of this study was to further clarify the mechanisms by which salt injury occurs, with emphasis on the water relations of seedlings treated with NaCl and Na_2SO_4. In the final chapter, the results of these studies have then been summarized, and the implications of these results to practical application have been discussed.

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Chapter II

Literature review

2.1 Biology of jack pine

Jack pine (*Pinus banksiana* Lamb.) is native to the Canadian boreal forest, with a range extending from Nova Scotia to northern British Columbia (Mirov, 1967). In western Canada its range overlaps that of lodgepole pine, with which it hybridizes freely forming a hybrid swarm in the region of central Alberta. In eastern North America, jack pine is found as far south as northern Illinois and New York, and it has been planted outside of its natural range in Alaska and the American Midwest. This species is the northernmost pine in Canada, and is well adapted to harsh environments. A high water content is normally maintained through the winter, and through periods of drought (Bliss and Mayo, 1980). However, pines are known to be tolerant of water loss from the protoplasm (Mirov, 1967), and may recover from shoot water potentials as low as –4.0 MPa (Bliss and Mayo, 1980). Although most of the root system is found in the top 0.5 meter of soil, the taproot may grow up to 3 meters to reach water deep in the soil (Rudolph, 1958), and roots may become encased in a suberized layer to conserve moisture through winter or summer drought (Mirov, 1967).

This pine is usually found on well-drained, fine to coarse sandy soils or loams (Cayford et al., 1967). It is a pioneer species, often establishing after fire in large stands of even age, and in the boreal forest may be succeeded by spruce and fir (Cayford et al., 1967). Jack pine may, however, be an ecological climax species on dry, nutrient-poor sites (Rudolf, 1958). In jack pine stands in the Athabasca region of northern Alberta the understory is dominated by lichens. Understory composition changes as the stand ages and thins, but other species rarely establish in the canopy in this area (Carrol and Bliss, 1982). Natural regeneration normally occurs after fire, which causes the serotinous cones to open, and results in pure stands of even age (Cayford et al., 1967). In reforestation, mechanical site preparation is used to expose mineral soil prior to planting or seeding. Mortality of planted seedlings is high in the first year as seedlings are susceptible to

water deficit stress, and growth and establishment rates may be improved by planting in sheltered locations. Jack pine has a rotation time of 50 to 80 years (Rudolph, 1958), and is commercially harvested for pulpwood, posts and railroad ties (Alberta Department of Energy and Natural Resources, 1977).

2.2 Composite tailings

The extraction of bitumen from the oil sands ore is accomplished using a hot water process, which generates a large volume of fine tailings that remain in suspension, presenting a challenge for reclamation. The composite tailings (CT) process, described by Syncrude Canada Ltd., involves the mixing of these fine tailings with coarser tailings, and adding a chemical coagulant to produce a non-segregating slurry (Matthews et al., 2000) (Fig. 2.2). A similar process is in use at a second mining operation, Suncor Energy, where it is referred to as consolidated tailings. The slurry is deposited in contained cells, where it releases a relatively solids-free water, which is then returned to the extraction plant. While a 30 – 60% of the water contained in the CT deposit will be released relatively rapidly (< 2 years), some will remain as pore water, and will likely be expressed over a number of years as the deposit continues to settle. Water released from the CT deposit will be referred to as "CT water" throughout this dissertation. CT water is relatively saline, with an electrical conductivity of > 4 mS cm^{-1} (MacKinnon et al., 2000), and recycling of the water is expected to result in increased salinity over time. The origin of the salts is from both the ore, which contains Na, Cl, and SO_4 in varying proportions, and from gypsum ($CaSO_4$) which is currently in use as the chemical coagulant (MacKinnon et al., 2000). At present, salinity occurs in a range that is potentially limiting to the growth of plants. CT water is somewhat alkaline (pH 7.8-8.5), and contains elevated levels of B, which could also inhibit plant growth. Current plans for the reclamation of CT deposits call for the establishment of terrestrial ecosystems on much of this land area. Although present plans call for a cap of sand and reclamation soil to be placed on the CT, plants may be exposed to CT water when it is displaced upwards by the settling deposit.

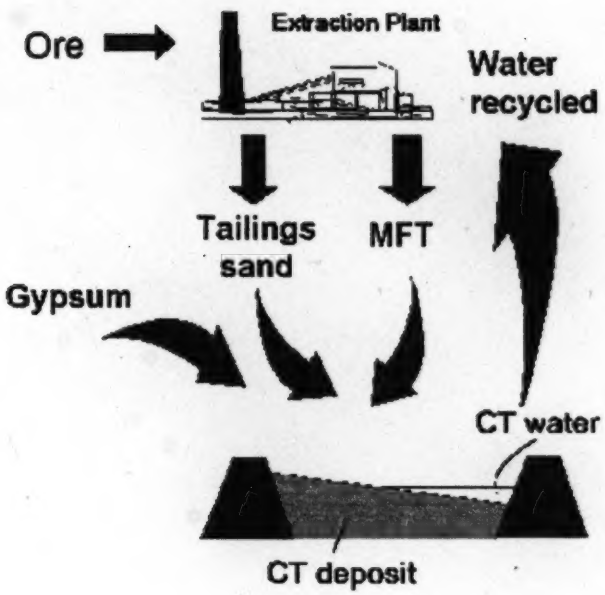

Fig. 2.2. Schematic diagram of composite tailings (CT) process. MFT – mature fine tails.

2.3 Salt stress in plants

Throughout the world salinity is a limiting factor to plant growth. Saline soils occur naturally, but increasing salinization of irrigated soils occurs when solutes in irrigation water are concentrated by evapotranspiration. Because of the large economic impact on agriculture, a large volume of research has addressed salt stress in plants, with the bulk of this work involving agricultural species. A few species, the halophytes, are adapted to growth under saline conditions. These plants maintain an osmotic gradient at high levels of external salinity by accumulating high tissue Na and Cl levels (Greenway and Munns, 1980). Excess salts may be excreted from the leaf by means of specialized salt glands in some species (Jacoby, 1994). Most species however, are classified as glycophytes, which are excluded from, or show reduced growth on saline soils. The direct effects of salts on plants are thought to be osmotic stress, and ion toxicity, which will be discussed in more detail. Salinity also induces secondary stresses including the inhibition of nutrient uptake and movement within the plant, resulting in nutritional disorders (Alam, 1994; Grattan and Grieve, 1999). Salinity may also reduce, or increase the ability of the plant to tolerate other environmental stresses, such as drought, hypoxia, or excess heavy metals (Singh et al., 1997). The observed effects of salinity on growth, photosynthesis and photosynthetic pigments, may be the result of direct stress, secondary stress, or altered hormonal balance.

A reduction in the content of photosynthetic pigments in the leaves has often been observed under saline conditions. Chlorophyll a content decreased in NaCl-treated tomato (*Lycopersicon esculentum* Mill.) plants (Khavare-Nejad and Mostofi, 1998), and chlorophyll a and b decreased in alfalfa (*Medicago sativa* L.) plants as concentration of NaCl treatment increased (Khavari-Nejad and Chaparzadeh, 1998). In woody plants, salt exposure does not typically result in chlorosis (Bernstein, 1975). The effect of salinity on carotenoid content varies greatly between species. Beta-carotene decreased in tomato plants treated with NaCl (Khavare-Nejad and Mostofi, 1998), but was not affected in alfalfa (Khavari-Nejad and Chaparzadeh, 1998). Carotenoid content increased in red and white cabbage *(Brassica oleracea* L.) and horsebean (*Canavalia ensiformis* (L.) DC.) under NaCl and Na_2SO_4 stress, but content decreased in red common perilla (*Perilla*

frutescens (L.) Britton) and did not change in cotton (*Gossypium hirsutum* L.) (Strogonov, 1973).

Reductions in photosynthesis have been measured in many species under saline conditions. Photosynthetic rates decreased in NaCl-treated alfalfa (Khavari-Nejad and Chaparzadeh, 1998), sapodilla (*Manilkara zapota* (L.) Royen) (Micklebart and Marler, 1996), and several poplar (*Populus* spp.) species (Fung et al., 1998), but were not inhibited by high levels of Cl or Na in olive (*Olea europa* L.) (Tattini et al., 1995). Reduced photosynthesis could result from decreased content of photosynthetic pigments, but photosynthetic rates were also found to decrease at NaCl levels where no chlorosis or enzyme inhibition occurred (Yeo and Flowers, 1986a). Injury to the photosynthetic mechanism may therefore be at the chloroplast level, as lamella and grana appeared disrupted in the cells of tomato plants treated with NaCl (Khavari-Nejad and Mostofi, 1998).

Ultimately, the energy produced by photosynthesis is used for maintenance, transport, storage, and growth, and the energy requirements of the first three are greatly increased under saline conditions (Cheeseman, 1988). Starch content of the stem, and leaf ATP content decreased as treatment concentration of NaCl increased in oak (*Quercus rober* L.) possibly due to use in Na exclusion (Alaoui-Sosse et al., 1998). Reduced growth could result from the diversion of resources to compartmentation of salt ions and synthesis of organic solutes (Volkmar et al., 1997), or may also result from reduced cell wall elasticity or an increased yield threshold (Cramer and Bowman, 1994). Changes in cell wall properties have been reported in salt-treated plants (Munns et al., 1983; Prichard et al., 1991; Neumann, 1993; Neumann et al., 1994). Cell elongation is also very sensitive to small changes in water potential (Pugnaire et al., 1994), which could also limit growth in moderately saline conditions. A reduction in cell size was thought to be an adaptation to NaCl in isolated tobacco (*Nicotiana tabacum* L.) cells (Hasegawa et al., 1986). Leaf area of kenaf (*Hibiscus cannabinus* L.) was reduced by NaCl treatment, and this was mainly due to a decrease in cell size (Curtis and Lauchli, 1987). However in NaCl-treated bean (*Phaseolus vulgaris* L.), leaves increased in thickness mainly due to increased length of palisade mesophyll cells (Longstreth and Nobel, 1979).

Salinity has been reported to affect hormonal concentrations in plants (Naqvi, 1994). Reduced leaf growth in bean was explained by leaf Na concentrations and increased abscisic acid (ABA) levels (Sibole et al., 1998). Increased ABA has also been found in the roots and leaves of citrange (*Citroncirus webberi* cv. Carrizo) (Gomez et al., 1998). Models of ABA movement in salt-stressed castor bean (*Ricinus communis*) suggest that this growth regulator could act as both a root-to-shoot and a shoot-to-root signal (Jeschke et al., 1997). In tomato, concentrations of ABA in the leaves were increased by salt treatment, while levels of indole-3-acetic acid (IAA) decreased in roots but were apparantly not the direct result of changes in root ABA concentrations (Dunlap and Binzel, 1996). Effects of ABA include stomatal closure, increased root-to-shoot ratio, increased permeability of roots to water (McKeon et al., 1995), and reduced cell elongation due to reduced cell wall plasticity (Shamshad and Naqvi, 1994). A number of studies have found some alleviation of salinity-induced growth reduction by treating plants with gibberellic acid or indole-acetic acid (Amzallag, 1997). Other possible signal transduction pathways involved in salt stress are the polyphosphoinositide system, or protein kinase C (Jaiwal et al., 1997).

2.4 Water deficit stress

Plants growing in a saline substrate are subjected to osmotic stress due to the low osmotic potential of the soil solution. In some species, osmotic stress may be the predominant factor limiting plant growth in saline soil. For instance, salt level had a much greater effect on shoot weight than did salt type in chickpea (*Cicer arietinum* L.) (Lauter and Munns, 1986). As occurs under drought stress, salinity often results in stomatal closure and reduced transpiration rates. Stomatal resistance has been found to increase in poplar (Fung et al., 1998), olive (Tattini et al., 1995), and celery (*Apium graveolens* L.) (Pardossi et al., 1998) under NaCl stress, and in loblolly pine (*Pinus taeda* L.) exposed to seawater (Johnson and Young, 1993). In bean (Sibole et al., 1998) and sycamore (*Acer pseudoplatanus* L.) (Khalil and Grace, 1993), increased stomatal resistance was related to increased concentrations of ABA in the leaves. Decreased transpiration rates have been measured in NaCl-treated radiata pine (*Pinus radiata* D.

Don) (Sands and Clarke, 1977). Although ABA levels increase in response to salinity, one recent study showed that water deficit stress was responsible for reduced transpiration rates (Myers et al., 1998). The hydraulic conductivity of roots has been found to decrease under NaCl salinity (Azaizeh et al., 1992; Evlagon et al., 1992), which could potentially affect transpiration rates. Root hydraulic conductivity is also reduced by deficiency of N or P (Carjaval et al., 1996) and is related to the density or activity of water channel proteins (Clarkson et al., 2000), through which water crosses the plasma membrane.

As the osmotic potential of the substrate decreases, uptake of water from the substrate slows as the water potential gradient from plant to soil decreases (Volkmar et al., 1997), and the water potential of the plant must also decrease for the plant to continue taking up water. Water potential decreased in NaCl treated poplar species (Fung et al., 1998) and the decrease was correlated with an increase in soil salinity in radiata pine (Myers et al., 1998). Although turgor may be temporarily reduced by salinity, with a corresponding reduction in growth, over the long term turgor potential must be maintained by a corresponding increase in cell wall elasticity (Neumann et al., 1988), or a decrease in the osmotic potential of the cells. Increases in the osmotic potential of the cytosol may be accomplished by the synthesis of neutral organic compounds such as proline, glycinebetaine, sucrose and fructose (Chandler and Thorpe, 1987; Chen et al., 1998), pinitol, sorbitol, and mannitol (Volkmar et al., 1997). In NaCl-treated tomato, sucrose was found to accumulate in both symplast and apoplast (Balibrea et al., 1999). While organic solutes were shown to make a significant contribution to solute potential in the roots of NaCl-treated corn (*Zea mays* L.) (Rodriguez et al., 1997), the contribution of organic compounds to osmotic adjustment is thought to be minimal in most species, in which inorganic ions are the primary means by which osmotic potential is reduced (Greenway and Munns, 1980).

2.5 Ion toxicity

Growth reduction could result in increasing concentrations of salt ions in plant tissue, if root-to-shoot transport rates of ions were maintained. However, growth reduction instead appears to be a consequence of tissue salt accumulation (Munns and Termaat, 1986), and shoot Na was correlated with dry weight in both NaCl- and Na_2SO_4-treated plants (Lauter and Munns, 1986). Leaf necrosis is often observed under conditions of moderate to severe salt stress, and is often attributed to ion toxicity. Injury to ponderosa pine (*Pinus ponderosa* P. Lawson & C. Lawson) from NaCl treatment was found to be greater than that from isoosmotic concentrations of Na_2SO_4, $CaCl_2$, or $MgCl_2$ (Spotts et al., 1972), and severe necrosis in this species under NaCl irrigation was attributed to ion toxicity rather than osmotic stress (Bedunah and Trlica, 1979). Necrosis has been related to foliar Na and Cl content in eastern white pine (*Pinus strobus* L.) (Hall and Hofstra, 1972) and red pine (*Pinus resinosa* Aiton) although in the latter, trees with high needle Cl varied widely in the amount of necrosis (Sucoff et al., 1975).

Both Na and Cl may be toxic at elevated levels in plant tissues. The SO_4^{2-} ion may also be toxic in plant tissues (Chandler and Thorpe, 1987), but its effects are not well known. The greater necrosis in highbush blueberry (*Vaccinium corymbosum* L.) treated with Na_2SO_4 than isomolar NaCl (Muralitharan et al., 1992) suggests that Na toxicity may be greater than Cl toxicity in this species. However, woody plants are susceptible to injury by either Na or Cl accumulation (Bernstein, 1975) and most woody plants tend to be more sensitive to excess Cl than to high levels of Na (Shannon et al. 1994). A synergistic effect between Na^+ and Cl^- has also been reported, with greater injury occurring when both ions are present (Spotts et al., 1972; Martin and Koebner, 1995), and a greater uptake of Na has been reported under NaCl salinity than under Na_2SO_4 salinity at the same treatment concentration of Na (Kahn et al., 1995; Renault et al., 2001). The means by which ions injure an intact plant, however, are as yet poorly understood. *In vitro* studies have shown protein synthesis and the activity of some enzymes to be inhibited by high levels of Na and Cl (Greenway and Munns, 1980), but such inhibition has not been demonstrated in intact plants. To the contrary, protein synthesis has been found to increase in salt-treated plants (Hurkman and Tanaka, 1987). Evidence suggests

that Na^+ and Cl^- ions may alter membranes, resulting in reduced selectivity. Sodium has been found to displace Ca^{2+} from the plasma membrane, and this is followed by K^+ efflux (Cramer et al., 1985). Chloride has been found to alter membrane lipid composition resulting in increased membrane permeability (Kuiper 1968), and this leakage has been proposed as a mechanism of Cl^- induced injury (Bernstein, 1975). Membranes have also been found to be more permeable to PO_4 in corn treated with NaCl + $CaCl_2$ (Roberts et al., 1984).

2.6 Ion transport

From the soil solution, water and solutes may flow through the root by either the apoplastic or symplastic pathway. At the endodermis, apoplastic flow is prevented by the casparian strip, and both water and solutes must cross the plasma membrane. Most solutes, however, enter the symplast at the root epidermis (Clarkson, 1988). The "single-equivalent membrane model" of water and solute transport (Dalton et al., 1975) assumes that the endodermis allows no bypass flow. A small amount of apoplastic flow does occur due to breaks in the endodermis, and the contribution of this pathway is considered in the more recent "composite transport model" (Steudle, 1995). Once across the endodermis, water and solutes enter the xylem where they are transported to the shoot in the transpiration stream.

The entry of cations into the cell is thought to be passive, occurring through ion channels which may have a low degree of selectivity (Yeo, 1998). A high selectivity for K over Na may be related to salt tolerance in some species, but not in others (Volkmar et al., 1998). Although Na influx is be passive, total Na uptake was 25% of influx, indicating that a substantial efflux occurred in NaCl-treated corn roots (Jacoby and Hanson, 1985). Na efflux is an active process, by means of a Na^+/H^+ antiport, which pumps Na out of the cytoplasm to either the apoplast or into the vacuole (Frommer et al., 1999). Synthesis of this antiport may be induced by NaCl (Blumwald and Poole, 1987). Expression of an identified Na^+/H^+ antiporter (AtNHX1) has recently been increased in transgenic lines of *Arabidopsis thaliana*, with the resulting plants showing increased

tolerance to NaCl (Apse et al., 1999). The greater shoot Na concentrations of these plants as compared to the wild-type indicates an increase in Na compartmentation.

The transport of anions across the plasma membrane into the cell appears to be an active process (Maas and Ogata, 1972). Cl^- enters the cell actively, possibly through a $Cl^-/2H^+$ symporter, but then may cross the tonoplast passively (Poole, 1988). Cl^- may be accumulated to high concentrations in the vacuole by entry through anion channels, with a driving force provided by H^+ pumps (Plant et al., 1994). Influx of SO_4 by active processes has been demonstrated in algae (Raven, 1988). A summary of ion transport is shown in Fig. 2.6.

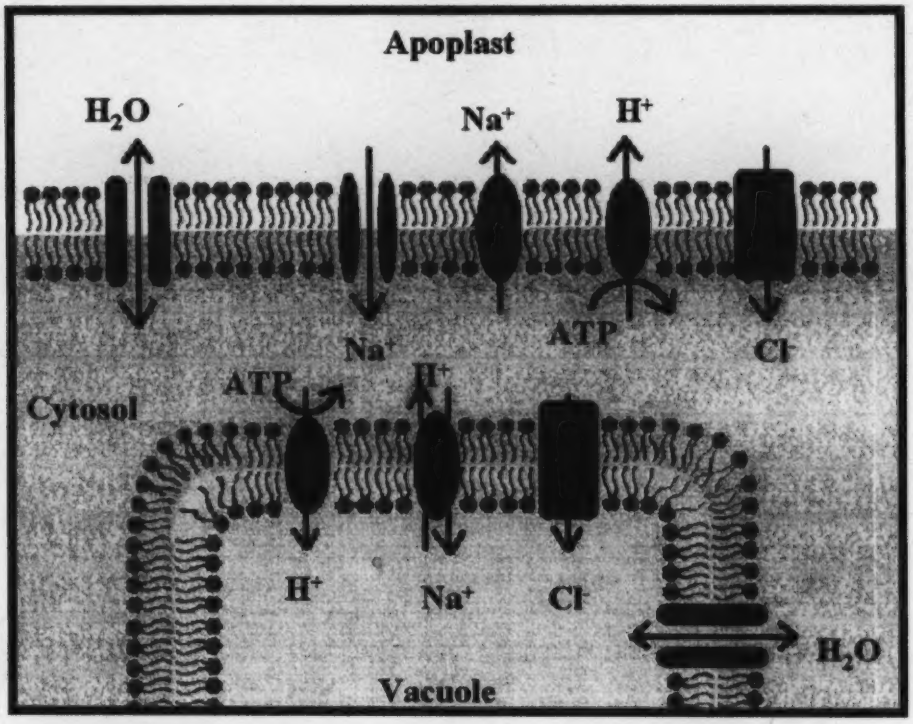

Fig. 2.6. Summary of water, Na^+, and Cl^- transport through the plasma membrane and tonoplast.

2.7 Salt tolerance and ion accumulation

In order to survive in saline environments, some species are able to restrict the uptake of salt ions, or restrict their translocation to the shoot. Radiata pine accumulated 6 to 10 times less Na than did eucalyptus (*Eucalyptus grandis*) receiving the same Cl salt treatment, and the authors concluded that the pine is able to limit uptake or translocation of Na (Myers et al., 1998). Rootstocks of trifoliata (*Poncirus trifoliata* (L.) Raf.) and sweet orange (*Citrus sinensis* L.) differed in leaf and stem Na and Cl, but not in root Na and Cl concentrations (Grieve and Walker, 1983) suggesting differences in their ability to restrict translocation. Other species that appear to retain Na in the roots include oak (Alaoui-Sosse et al., 1998), sorghum (*Sorghum bicolor* L.) (Kahn et al., 1995), castor bean (Jeschke and Pate, 1991) and dogwood (*Cornus stolonifera* Michx.) (Renault et al., 2001). Sodium and Cl may be compartmentalized in root of olive, but were quickly re-distributed when NaCl stress was released and transpiration resumed (Tattini et al., 1995). Sodium may also be returned to the roots via the phloem and excreted, as demonstrated in bean (Jacoby, 1979). Other species seem to lack the ability to restrict translocation of Na and Cl to the shoot. In highbush blueberry (Muralitharan et al., 1992) and poplar (Fung et al., 1998), Na accumulated to a greater extent in the leaves than in the roots, and the distribution of Cl was found to be fairly uniform in *Leucaena leucocephala* (Lam.) de Wit (Jaenicke et al., 1996) and in radiata pine treated with NaCl (Sands and Clarke, 1977).

Some NaCl-tolerant tree species such as red oak (*Quercus rubra* L.) and white oak (*Q. alba* L.) accumulated little Cl in tissue, while other species such as paper birch (*Betula papyrifera* Marshall) appeared to tolerate high tissue Cl content (Shortle and Rich, 1970). Potentially toxic ions reaching the shoot must be stored where they cannot damage the photosynthetic machinery. In woody plants, stems provide a large amount of tissue in which ions could potentially be sequestered. Sodium was found to accumulate in the stems of sweet orange until their capacity was exceeded at concentrations of around 90 – 100 mmol/kg dry weight (Boland et al., 1997). Radiata pine also appears to store Na in the stems, as tissue Na increased more in stems that in leaves and roots following treatment with NaCl (Sands and Clarke, 1977). Sodium may also be

selectively transported to older leaves, where it can potentially be lost through leaf abscission. Although tomato accumulates Na in the leaves, tolerance seems to be related to partitioning within the shoot, as Na concentrations were lower in young leaves of tolerant plants (Cuartero and Fernandez-Munoz, 1999). High necrosis and Na accumulation was observed in the first flush leaves but not in second flush leaves of NaCl treated oak (*Quercus rober* L.) (Alaoui-Sosse et al., 1998).

Within a tissue, salt ions could accumulate in the apoplast, where they would draw water out of the cells by osmosis resulting in cell dehydration (Oertli, 1968). Measurements of ion concentrations in the apoplast or cytosol are difficult, and the evidence to date is conflicting. Chloride ions were found to accumulate in both cell walls and vacuoles of radiata pine (Foster and Sands, 1977), and NaCl concentrations in the apoplast of rice (*Oryza sativa* L.) leaves were calculated to be 600 mM under moderate salinity (Flowers et al., 1991). However, the hypothesis that ions are compartmentalized in the vacuole, thereby maintaining low concentrations in the cytosol, is widely accepted (Volkmar et al., 1998). In NaCl-treated *Plantago*, the more salt tolerant *P. maritima* was able to pump Na^+ into the vacuole, while the less salt tolerant *P. coronopus* was not (Prins, 1995). The maintenance of growth under saline conditions can provide new tissue in which to compartmentalize salt ions (Volkmar et al., 1998). If growth is insufficient in this respect, ion accumulation will eventually result in death (Munns and Termaat, 1986). Some recent evidence suggests that salt accumulation in the shoot is determined not by biochemical properties, but by physical properties such as transpiration (Dalton et al., 2000).

2.8 Salinity type

The majority of studies have investigated the effects of a single salt, NaCl, on plant growth and physiology. A large volume of work in the mid-1900's investigated the effects of salts containing Na, Mg, Cl and SO_4 on a variety of crop plants, and found that these salts differed in their effects on plant growth, pigment content, organic solutes, and amino acids (Strogonov, 1964). Since then, only a few studies have focussed on the

effects of the two most commonly found salts in saline soils around the world, NaCl and Na_2SO_4. Growth reduction and injury was reported to be greater in NaCl than in isomolar Na_2SO_4 in potato (*Solanum* spp.) (Bilski et al., 1988) and sorghum (Kahn et al., 1995), and needle injury in ponderosa pine was greater as a result of NaCl-treatment than treatment with Na_2SO_4 (Spotts et al. 1972). Conversely, the adverse effects of salinity on red-osier dogwood (Renault et al., 2001) and blueberry (Muralitharan et al. 1992) were greater in Na_2SO_4 than by isomolar NaCl. Early seedling growth was found to be more affected by Na_2SO_4 than by isomolar NaCl in jack pine, but spruce species were similarly affected by the two salts (Croser et al., 2001). Nutritional effects have been shown to differ between the two salts. Treatment of barley roots with 30 mM Na_2SO_4 inhibited the translocation of Ca more than did treatment with 60 mM NaCl (Halperin et al., 1997), and less loss of K was found in sorghum treated with Na_2SO_4 than with NaCl (Kahn et al., 1995).

2.9 Nutrient deficiency

While nutrient deficiency is unlikely to affect plants in experiments of relatively short duration, it may be a factor in plants exposed to salinity over a longer time period (Munns and Termaat, 1986). High levels of Na in the substrate most often result in a high ratio of Na to Ca and Mg (sodium adsorption ratio: SAR). The imbalance of cations in the soil leads to cation imbalances in the plant (Bernstein, 1975), most often expressed as a deficiency of Ca, Mg, or K (Alam, 1994).

Within the plant, Ca plays important roles in signal transduction and membrane integrity. High levels of Na may reduce the availability of Ca in the substrate, however reported effects of salinity on tissue Ca concentrations are variable. Reduced tissue Ca content following NaCl treatment has been reported for roots and shoots of sesame (*Sesamum indicum* L.) (Yahya, 1998), leaves of sapodilla (Micklebart and Marler, 1996), and roots and stem of maritime pine (*Pinus pinaster* Ait.) (Saur et al., 1995). Conversely, Ca concentrations increased in roots and leaves of NaCl treated bean (Carbonell-Barraching et al., 1997), and increased with increasing treatment concentration of Na_2SO_4

in barley (*Hordeum vulgare* L.) (Janzen and Chang, 1987). The addition of Ca to the substrate may alleviate salinity effects in some species (Grattan and Grieve, 1999).

Although Mg is an element essential to plant function, Mg nutrition under saline conditions has received relatively little attention. As with Ca, changes in tissue Mg content resulting from NaCl treatment vary between species. Tissue Mg was reduced in roots and shoots of sesame (Yahya, 1998) and in roots and stems of maritime pine (Saur et al., 1995), increased in leaves of NaCl treated bean (Carbonell-Barraching et al., 1997), and was unaffected in sapodilla leaves (Micklebart and Marler, 1996) and radiata pine needles (Sands and Clarke, 1977; Myers, et al., 1998).

Potassium plays an important role in osmoregulation, and although plants are highly selective for K uptake, reductions in tissue K are frequently reported under high Na conditions (Grattan and Grieve, 1994). Efflux of K occurs when membrane selectivity is reduced due the displacement of Ca from the membrane (Cramer et al., 1985). Reduced tissue K has been found in shoots of sesame (Yahya, 1998), oak (Alaoui-Sosse et al., 1998), sapodilla (Micklebart and Marler, 1996), and bean (Carbonell-Barraching et al., 1997) treated with NaCl. However, K concentrations in *Leucaena leucocephala* (Lam.) de Wit (Jaenicke et al., 1996), almond (*Prunus amygdalus* L.) (Noitsakis et al., 1996), and poplar (Fung et al., 1998) were relatively unaffected by NaCl treatments. The maintenance of a low shoot Na/K ratio has been implicated as a mechanism of salt tolerance (Saur et al., 1995). Castor bean treated with NaCl was found to have increased translocation of K to young tissues (Jeschke and Wolf, 1988). However, Na/K ratio was not found to be related to salt tolerance in two mango rootstocks (*Mangifera indica* L.) (Schmutz and Ludders, 1998).

Studies of N nutrition in salt-stressed plants show much variation between species, but most have observed reduced shoot N in plants treated with NaCl (Grattan and Grieve, 1999). Nitrate reductase activity was found to be inhibited in roots of *Arthrocnemum fruticosum* (L.) under NaCl salinity (Eddin and Doddema, 1986). Reductions in N uptake may be due to competitive interactions between Cl^- and NO_3^- ions. Reports of reduced N in shoot tissue are mainly for agronomic species such as bean (Carbonell-Barrachina et al., 1997) and soybean (*Glycine max* L.) (Grattan and Maas, 1988), but few studies have investigated N uptake in woody species. Those that have,

show little change in shoot N (Sands and Clarke, 1977; Micklebart and Marler, 1996) or increased shoot N (Saur et al., 1995).

As with other nutrients, the effects of salinity on tissue P are highly variable between species. Shoot P increased in Canary Island pine (*Pinus canariensis* C. Sm.) (Tausz et al., 1998) and soybean under saline conditions (Grattan and Maas, 1988), and increases in root P were implicated in growth reduction of corn roots (Roberts et al., 1984). In soybean treated with NaCl, injury was attributed to the toxicity of high P and Cl content (Grattan and Maas, 1985). Reductions in tissue P following NaCl treatment have been reported for leaves of sapodilla (Micklebart and Marler, 1996), roots of maritime pine (Saur et al., 1995), and leaves of bean (Carbonell-Barrachina et al., 1997). Different salinity levels may produce different effects, as P was reduced in roots and shoots of sesame, treated with 10 and 20 mM NaCl, but increased in plants treated with 40 mM NaCl (Yahya, 1998). Different salts may also differently affect P nutrition, as P decreased in roots of radiata pine treated with NaCl, but showed large increases in roots treated with $CaCl_2$ (Sands and Clarke, 1977).

The effects of salinity on S nutrition have been reported in very few studies. In sorghum treated with Na_2SO_4, tissue SO_4 concentrations increased with increasing treatment concentrations (Kahn et al., 1995), and a mechanism of Na_2SO_4 resistance in lucerne (*Medicago sativa* L.) may be to restrict S entry to shoot (Rogers et al., 1998). Little is known of S uptake under NaCl salinity, but S concentrations did not change in leaves of NaCl treated sapodilla (Micklebart and Marler, 1996).

Salinity may reduce the solubility of several micronutrients, however, plant uptake of micronutrients is highly variable (Grattan and Grieve, 1994). Treatment with NaCl was found to increase Mn concentrations in leaves of sapodilla, while concentrations of Cu, Fe, and Zn were unaffected (Micklebart and Marler, 1996). In roots and shoots of NaCl-treated tomato, soybean and squash (*Cucurbita pepo* L.), Fe and Zn concentrations increased (Maas et al., 1972). Concentrations of Mn were also found to increase, with the exception of tomato roots, in which decreased Mn was reported.

2.10 Tolerance, genetic variability and acclimation

Many previous studies have reported differences in plant responses and salt tolerance between species and between accessions or cultivars within a species. For instance, different lines of lucerne vary in response to NaCl and Na_2SO_4 (Rogers et al., 1998). Characteristics of plants to tolerate salinity include the ability to regulate osmotic potential, and the ability to sequester Na in the vacuole (Jacoby, 1994). Although osmotic stress is a direct result of salinity, drought tolerance was not found to be related to salt tolerance in potato species (*Solanum* spp.) treated with NaCl or Na_2SO_4 (Bilski et al., 1988).

Differences in tolerance may be related to the ability of the roots to restrict the translocation of salt ions to the shoots. Citrus (*Citrus reticulata* Blanco, *C. sinensis*, and *Poncirus trifoliata*) rootstocks were found to differ in leaf and stem Na and Cl, but not in root Na and Cl concentrations, and leaf Cl was not related to salt tolerance in these species (Grieve and Walker, 1983). Sodium transport from root to shoot was restricted in salt tolerant olive genotype, and large genotypic differences in transport rates were found (Tattini, 1994). A salt-tolerant mango rootstock was found to restrict Na translocation to the shoot and tolerate higher Cl levels in leaves (Schmutz and Ludders, 1998), and an isolated salt-tolerant cell line from orange (*Citrus sinensis* L.) was found to have lower Na and Cl uptake than non-tolerant cells (Ben-Hayyim and Kochba, 1983). Genotypic differences in Na uptake have been described in almond (Noitsakis et al., 1996), rice (*Oryza sativa* L.) (Yeo and Flowers, 1986a). Such differences could result from differing apoplastic flow, as individual variation in amount of apoplastic flow results in differences in shoot Na accumulation in rice (Yeo et al., 1987). In addition to limiting salt uptake or translocation, differences in salt tolerance could potentially result from a number of other factors. Yeo and Flowers (1986b) hypothesized that genotypic variation could be due to differences in the ability to maintain low salt levels in the apoplast. Genetic variation in ion compartmentation (Yeo and Flowers, 1986a) has been demonstrated in rice, and improved compartmentation is associated with salt tolerance (Yeo, 1998).

Exposure to salinity may also result in physiological changes that increase the tolerance of the plant to further salinity. Such an increase in tolerance can be measured

as a restoration of relative growth rates, or as an increase in the level of salinity at which the life cycle can be completed (Poljakoff-Mayber and Lerner, 1994). Acclimation to NaCl salinity was induced in sorghum (Amzallag, 1994), and was dependent on the length of the treatment period (Amzallag et al., 1990). ABA signals are involved in physiological changes in plants, and increases in ABA were related to a greater restriction of Na and Cl accumulation in bean (Montero et al., 1997). An increased ability of cells to sequester salt ions in the vacuole could convey salt tolerance. Roots of barley showed an increase in vacuolar H^+ pumps under saline conditions (Zhang et al., 1998). The acclimation of cotton to salinity involved osmotic adjustment primarily by the accumulation of Na and Cl (Plaut and Federman, 1991).

2.11 Salinity effects on coniferous species

Although the bulk of salinity research has concentrated on crop plants, some studies have investigated the effects of salts on conifers, which may be exposed to salts through road salt application or seawater inundation. Needle necrosis is commonly reported in salt-treated conifers, but the mechanism of injury is unknown. Anatomical observations of salt-treated white spruce (*Picea glauca*) could not discern whether injury was due to desiccation or other factors (Kutscha et al., 1977).

Some pine species appear to be relatively tolerant of salts. Neither leaf elongation rate or stem diameter of radiata pine was affected by irrigation with saline effluent up to 5 dS/m (Myers et el., 1998), and in maritime pine, growth was reduced only by NaCl treatments of 150 mmol or higher and no needle necrosis was evident on plants treated with 250 mM NaCl (Saur et al., 1995). Loblolly pine had less observable injury as a result of seawater flooding than did slash pine (*Pinus elliottii* Engelm.) (Land, 1974). Severe necrosis was observed in NaCl-irrigated ponderosa pine, and was attributed to ion toxicity rather than osmotic stress (Bedunah and Trlica, 1979). Needle tip and intercostal necrosis was noted in salt injured Scotch pine (*P. sylvestris* L.), with new needles and mesophyll cells showing the first signs of injury (Stewart et al., 1973). In a study of Cl distribution in radiata pine, Cl was found to be deposited preferentially in mycorrhizae, in

the outer cortex of roots, and in the lumen and cell wall of tracheids (Foster and Sands, 1977). Within the needles, Cl accumulated in epidermal cells and mesophyll cells walls. Within cells, most of the Cl was found in the vacuole, not cytoplasm. This indicates compartmentalization within cells and tissues.

Studies with pine suggest that some species may be susceptible to ion toxicity, as correlations have been found between needle necrosis and needle Cl and Na concentrations (Hall and Hofstra, 1972; Spotts et al., 1972; Sucoff et al., 1975). Interestingly, trees with high needle Cl varied widely in amount of necrosis, and high variability in conifer response to salt has been reported by other researchers (Bedunah and Trlica, 1979; Renault et al., 1998). Variation in salt sensitivity and Na accumulation in needles has also been found between provenances of maritime pine (Saur et al., 1995). It is possible that the large genome size of conifers may be an adaptation that enables these species to tolerate environmental stresses (Newton et al., 1994). Nutritional effects of salts on conifers may differ from the effects seen in agronomic species. In contrast with most crop species, K increased in needles of radiata pine treated with NaCl (Sands and Clarke, 1977; Myers et al., 1998) and increased in needles and stem of maritime pine treated with 250 mmol NaCl (Saur et al., 1995). No literature could be found on the effects of salts on jack pine.

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Chapter III

Saline pre-treatment of jack pine seedlings for planting on reclamation sites impacted by saline tailings[1]

3.1 Introduction

The Athabasca oil sands in northern Alberta, Canada, cover a total land area of 40,000 square kilometers, and contain bitumen, which is currently recovered by surface mining. Two large-scale commercial mining operations (Suncor Energy Inc. and Syncrude Canada Ltd.) are currently in production, and further development is underway. Current tailings management includes the containment of tailings within settling basins, and the production of a material known as consolidated (Suncor) or composite (Syncrude) tailings (CT) (Matthews et al., 2000). This involves mixing the sand fraction of the extraction tailings with mature fine tailings, and addition of gypsum at a rate of 750-1200g/m^3 as a chemical coagulant. The resulting non-segregating material is deposited in holding cells as slurry. This material is expected to de-water relatively rapidly, but pore water will continue to be expressed over a number of years as the deposit settles further. When the deposit becomes trafficable, plans are to reclaim the land to a mosaic of self-sustaining ecosystems, a large portion of which will be productive native forest. The tailings and associated waters that are currently being produced are relatively saline, with the main ions being Na^+ and Cl^- from the ore, and SO_4^{2-} from the added gypsum (MacKinnon et al., 2000). The relative proportions of these ions vary with variations in ore and processing, and salinity is expected to increase over time due to recycling of the process water. In addition to salinity, other chemical properties of CT that may affect plant growth include alkalinity and elevated levels of boron.

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Jack pine (*Pinus banksiana* Lamb.) is native to the Canadian boreal forest, with a range extending from Nova Scotia to northern British Columbia. Jack pine is an early-successional species that is typically found on nutrient-poor sandy soils (Cayford et al., 1967), and may therefore be a suitable reclamation species for these sites. Because jack pine is a dominant tree of mesic to xeric sites in the pre-disturbance ecosystem, and has potential commercial value, the re-establishment of this species is highly desired. Once established, reclamation sites may appear similar to natural stands, as these tend to be even-aged and low in diversity. Previous studies of conifer species have found that while lodgepole pine (*Pinus contorta* var. *latifolia*), white spruce (*Picea glauca*) and black spruce (*Picea mariana*) are only moderately tolerant of CT water, there was a large amount of individual variation in response (Renault et al., 1998a). Much of the reclamation of these mine sites will likely involve the planting of nursery-grown seedlings; however, previous results suggest that high mortality rates will result in high reclamation costs.

Pre-treatment of young seedlings prior to planting may result in increased survival rates of seedlings planted on reclamation sites. In order to test this hypothesis, jack pine seedlings that had survived a previous treatment with saline solutions were planted on a test deposit of composite tailings at Syncrude Canada Ltd., and monitored over two years. A parallel experiment was conducted in a controlled environment chamber to allow the effects of water chemistry to be distinguished from the effects of other environmental variables.

3.2 Materials and Methods

3.2.1 Plant material

Seeds were collected in October of 1997 from three jack pine stands. One stand is located on the Syncrude site (56°56.06N 111°31.95W), another near Suncor (57°05.95N 111°38.90W), and the third near Smoky Lake, Alberta (54°06.88N 112°10.38W). Seedlings were grown in foam seedling blocks (Beaver Plastics, Edmonton, Alberta:

160/60 ml) filled with quartz/feldspar sand (porosity of 28%) that had been washed with deionized water to eliminate silt and ions. Plants were grown in a controlled environment chamber at 70% relative humidity, 24°/18° C (day/night) temperature and 18 hour photoperiod, and watered daily with a nutrient solution (80 mg L^{-1} N, 60 mg L^{-1} P, 104 mg L^{-1} K, 100 mg L^{-1} Ca, 60 mg L^{-1} Mg, 79 mg L^{-1} S, 3 mg L^{-1} Fe, 0.40 mg L^{-1} Mn, 0.25 mg L^{-1} B, 0.14 mg L^{-1} Zn, 0.50 mg L^{-1} Cu, and 0.10 mg L^{-1} Mo, pH 5.0 to 5.3) recommended for pine seedling production (Wood 1995).

3.2.2 Pre-treatment

At 30 days of age, seedling blocks were placed in trays of distilled water with nutrients (control), CT release water with nutrients, or distilled water with nutrients and various concentrations of salt (30mM NaCl, 60mM NaCl, 30mM Na_2SO_4, or 60mM Na_2SO_4) for a period of two weeks. The chemical composition of CT water was analyzed by Syncrude (as described in chapter 4), and the major ions are reported in Table 3.2.2. The Na^+ level is approximately equivalent to 39mM, and Cl^- is approximately 15 mM. Boron and fluoride were present in concentrations of 3.5 mg L^{-1} and 2.1 mg L^{-1}, respectively, and the pH was 9.05.

Three replicate trays were used for each treatment, containing between 150 and 216 plants per tray. The greater numbers of seedlings were used for the highest salt pre-treatments, where high mortality was anticipated. Mortality was monitored over the treatment period, and over the following 30 days. A styrofoam block containing 24 seedlings per treatment combination (seed source x pre-treatment) was harvested, and freeze-dried prior to measurement of dry weight and ion analysis. Four seedlings from each treatment combination were randomly selected for ion analysis. Shoot and root Na and K contents of freeze-dried tissue were determined by atomic absorption after digestion with sulfuric acid and hydrogen peroxide at 350° C. The sand was then flushed with deionized water, and returned to the previous daily watering schedule with nutrient solution. Dormancy was induced, and from 20 to 28 weeks of age, seedlings were stored at 3° C in darkness.

Table 3.2.2. Major ions present in CT release water from pond 5 of Syncrude's 1995 NST Field Test that was based on gypsum treatment.

Cations	Concentration ($mg\ L^{-1}$)	(mM)	Anions	Concentration ($mg\ L^{-1}$)	(mM)
Na^+	904	39.3	F^-	2.1	0.11
K^+	25.6	0.65	Cl^-	490	13.8
Mg^{2+}	23.8	0.98	SO_4^{2-}	1300	13.5
Ca^{2+}	64.3	1.60	HCO_3^-	450	7.14

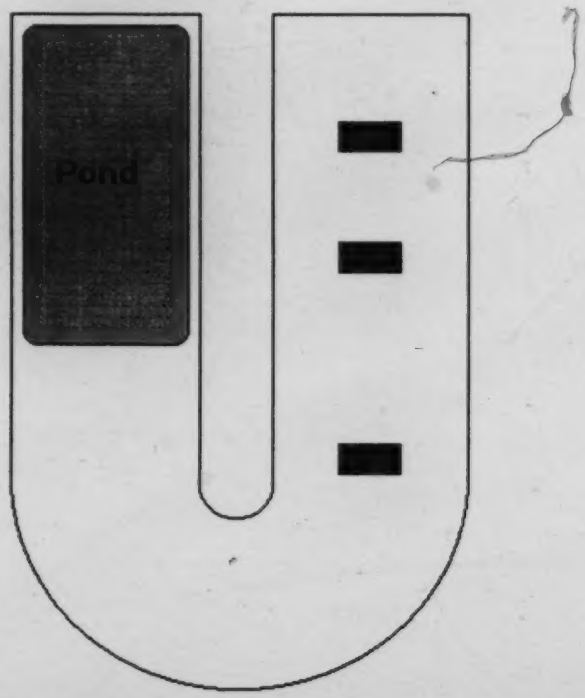

Fig. 3.2.3. Location of three planting sites (black rectangles) on a test CT deposit, located on the mine site of Syncrude Canada, in Fort McMurray, AB. The pond is shown as it appeared at the time of planting, in June of 1998.

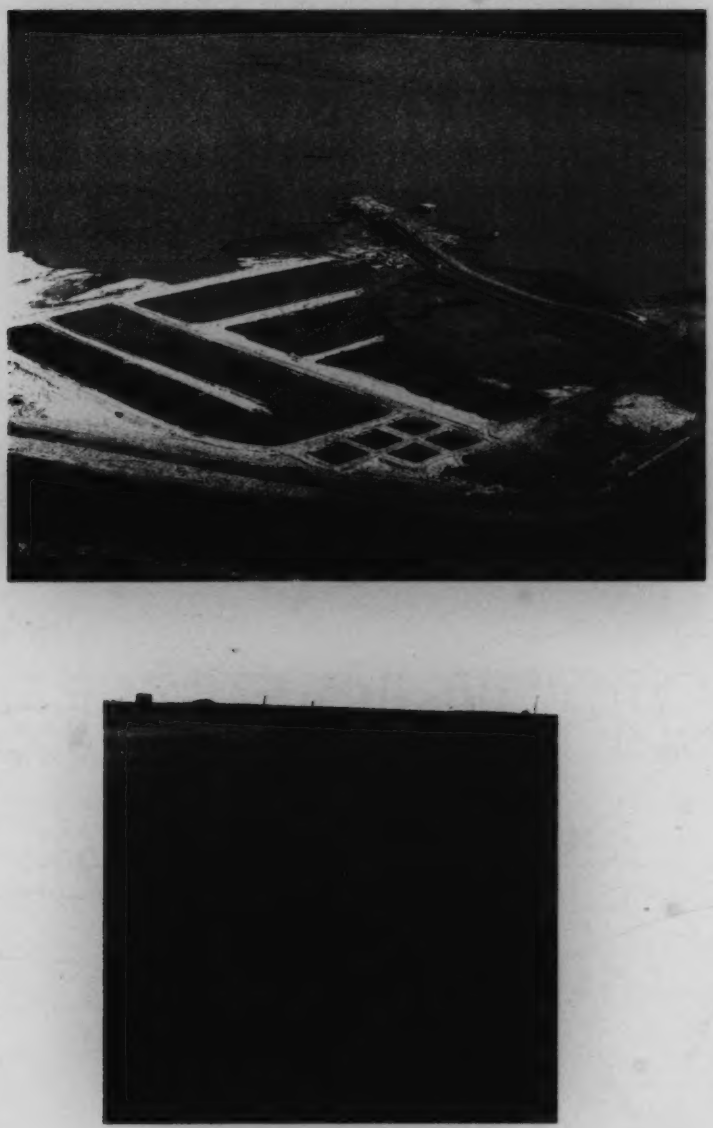

Fig. 3.2.4. Photograph of U-shaped test CT deposit at Syncrude Canada (top, photo courtesy of Syncrude Canada), and rows of jack pine seedlings planted on the deposit in 1998 (bottom).

3.2.3 Field experiment

In June 1998, seedlings were removed from cold storage, and one third of these from each pre-treatment group were randomly assigned to the field experiment. Three 10m x 10m plots were randomly located along the center line of the dry half of a test CT deposit, which was deposited in a lined, U-shaped cell at Syncrude in 1995 (Fig. 3.2.3). The CT material was dense and homogenous, with a hard surface crust. The plots were rotor-tilled to break the surface crust, then lightly compacted prior to planting. Irrigation was provided six times during the first month after planting, with water that collected in the lower end of the U-shaped cell. In each plot, between 145 and 150 seedlings were planted in rows 1.5 m apart, with a spacing of 3 plants m^{-1} within the rows. Survival and the flushing of apical buds were recorded in July and August of 1998, one and two months after planting, and in June and August of 1999. Seedling height was measured prior to removal from cold storage, and again in August of 1999.

3.2.4 Growth chamber experiment

A second experiment was performed in a controlled environment chamber to separate the effects of CT water chemistry from other environmental variables. Seedlings were removed from cold storage and placed in trays of treatment solution containing nutrients and 60 mM NaCl, 60 mM Na_2SO_4, CT water, or de-ionized water (control). Plants were removed from treatment trays for 24 of every 48 hours to avoid flooding stress. The sand was flushed with deionized water weekly, at which time treatment solutions were replaced. Environmental conditions within the chamber were as described above. A total of 1704 seedlings were used in a completely randomized block, full factorial experiment with three replicate trays per treatment. Factors considered were seed source (3), pre-treatment (6) and treatment (4), for a total of 72 treatment combinations. The number of seedlings per treatment combination varied due to differences in survival of the pre-treatment groups, but was a minimum of 8 plants. Survival and terminal bud flushing were monitored every 4 days for a period of 10 weeks, then all shoots were weighed and measured.

3.2.5 Data analysis

Data were analyzed using a general linear model (GLM) (Appendix B). The means were compared using the Duncan's multiple range test, and were considered significantly different at $\alpha = 0.05$. All data were analyzed using "SPSS 8.0" statistical software package (SPSS Inc., Chicago, IL).

3.3 Results

3.3.1 Pre-treatment

Seedling mortality was approximately 40% in the 60 mM Na_2SO_4 treatment, 12% in the 30 mM Na_2SO_4 treatment, and less than 5% in all other treatments. Seedlings from different seed sources did not differ significantly with respect to any of the measured parameters (Appendix B), and were combined for further analysis. Biomass of surviving seedlings was similarly reduced by all salt treatments with the exception of 30 mM Na_2SO_4 (Fig. 3.3.1). Sodium accumulation was greater in both roots and shoots of plants treated with 60 mM NaCl compared with those treated with 60 mM Na_2SO_4 (Table 3.3.1). Shoot and root K levels were slightly reduced by both of these treatments.

Table 3.3.1. Tissue Na and K content (mg g^{-1} dry weight) of jack pine seedlings treated beginning at 30 days of age (n = 12). A 14-day treatment with 60 mM Na_2SO_4 or 60 mM NaCl was followed by a 30-day recovery period in nutrient solution. Standard errors are shown in parentheses. Different letters indicate a significant difference between treatments at $\alpha = 0.05$.

		Control	60 mM Na_2SO_4	60 mM NaCl
Na^+	Shoot	0.20 (0.06) a	2.51 (0.42) ab	4.53 (1.17) b
	Root	0.47 (0.11) a	4.11 (0.46) bc	5.69 (1.62) c
K^+	Shoot	16.25 (1.00)	16.01 (1.35)	14.93 (1.23)
	Root	23.33 (0.89)	18.62 (1.84)	19.27 (1.47)

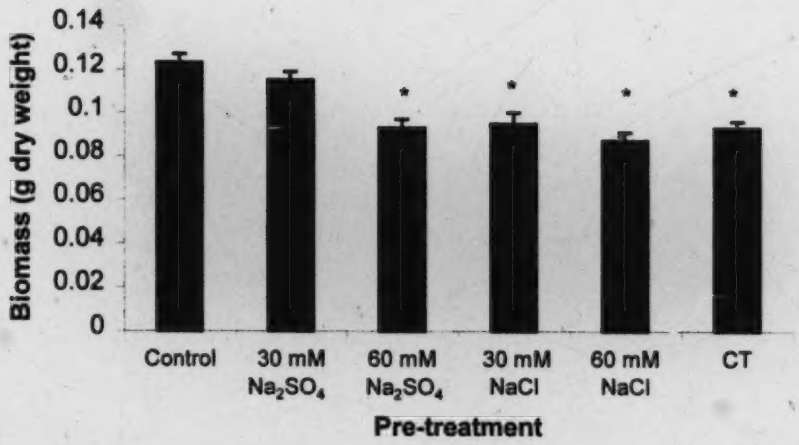

Fig. 3.3.1. Total biomass of jack pine seedlings after treatment for 2 weeks with salt solution. Bars represent standard error. * indicates a significant difference from control at $\alpha = 0.05$.

3.3.2 Field experiment

Seedlings from different seed sources did not differ significantly with respect to any of the measured parameters, and were combined for further analysis (Appendix B). Therefore, only pre-treatment was considered as a factor in the analysis. Seedling survival over the first growing season was greater than 90%; however, some needle injury was evident. Winter mortality was greater than 50%, and survival at the end of the second growing season (Aug. 1999) averaged 6.4%. Burial by blowing sand was a frequent cause of seedling mortality, and affected all pre-treatment groups equally. In a small number of seedlings (> 2%) mortality could be attributed to other mechanical injury, and no damage due to insects or rodents was apparent. A main cause of mortality within the growing seasons appeared as a drying and browning of the tissues. Seedling survival increased with increasing concentration of the treatment solution (Fig. 3.3.2.1), and was significantly greater in seedlings that had been treated with 60 mM NaCl than in controls.

Approximately 35% of all seedlings exhibited flushing of the terminal bud during the first growing season (Fig. 3.3.2.2). No significant differences were found between the flushing of control and treated seedlings; however, there was a clear trend of earlier flushing in salt-treated seedlings. Height growth at the end of the second growing season followed the same trend as survival, with seedlings receiving CT water and 60 mM NaCl pre-treatments showing heights of 7.4 cm and 7.3 cm, respectively, compared to 4.7 cm in control plants; however, the difference was not statistically significant.

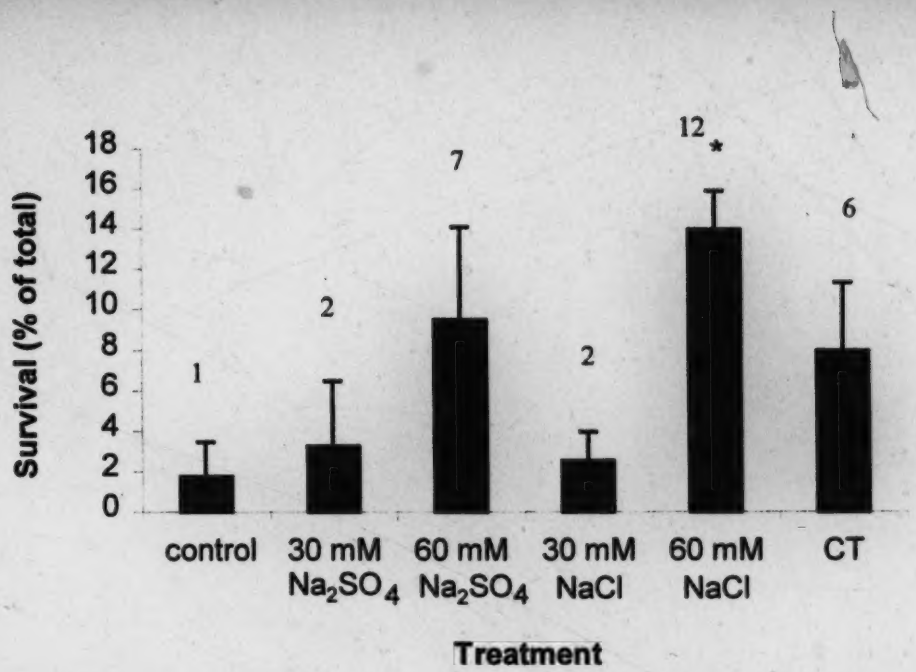

Fig. 3.3.2.1. Survival, after two growing seasons of jack pine seedlings treated with salt solutions prior to planting. * indicates a significant difference from control at $\alpha = 0.05$.

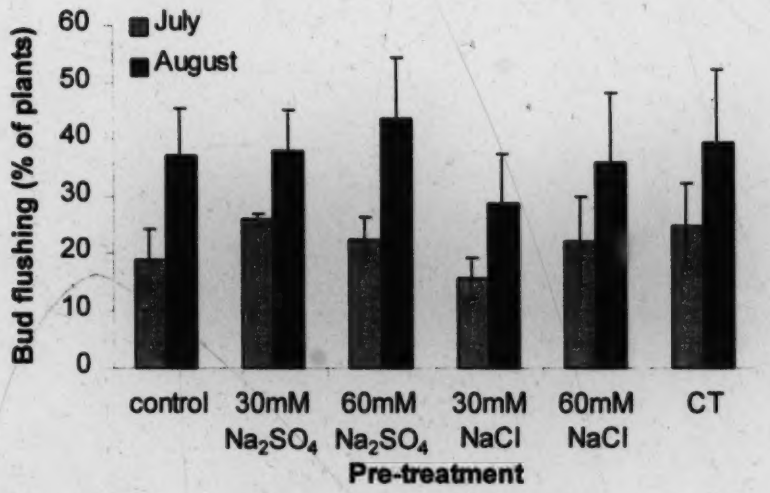

Fig. 3.3.2.2. Flushing of the terminal bud over the first growing season in jack pine seedlings treated with salt solutions at 30-days-of-age. Bars indicate standard errors.

3.3.3 Growth chamber experiment

Pre-treated seedlings did not differ from control seedlings with respect to height or bud flushing. Survival was greater than 95% in all treatments. Fresh weight was significantly reduced in two treatment combinations; 2.78 ± 0.12g in CT water/ CT water, and 2.36 ± 0.08g in 60 mM NaCl/ 60 mM Na_2SO_4 (pre-treatment/treatment) as compared to 4.77 ± 0.14g in controls (Appendix B). Although seed sources differed significantly with respect to the time of bud flushing, stem elongation rate, shoot fresh weight, they did not differ in response to treatment at the $p \leq 0.05$ level.

3.4 Discussion

In the field experiment, survival by the end of the growing season was very low. Winter mortality was high, and was followed by high mortality over the summer of 1999. Because plants in the parallel growth chamber experiment had very high survival rates when exposed to CT water for 10 weeks, water chemistry alone was not the main cause of mortality in the field. Plants exposed to CT water in the greenhouse did show visible injury over the growing season, suggesting that water chemistry contributed to plant stress in the field. The site received very little summer rainfall and temperatures on the bare surface of the deposit were sometimes as high as 45°C. A moderate to strong wind frequently blew fine sands and silts across the surface of the deposit, abrading the surface of needles, and contributing to water deficit stress. Jack pine on adjacent plots showed low transpiration rates and high diffusive resistance, indicating stomatal closure, while species that were able to maintain transpiration showed high survival rates (Renault et al., 1998b). Drought stress was, therefore, a likely cause of mortality, and irrigation in the month following planting may have accounted for the high survival rate during the first growing season.

Reclamation plans for CT deposits call for the placement of a capping material of tailings sand or overburden (minimum 1-m thick), plus a thinner layer of reclamation soil

prior to planting. The direct planting of seedlings on the deposit was used to allow a rapid assessment of effects of CT on jack pine establishment. While direct seeding is probably not a likely reclamation procedure on these sites, successful seed reproduction will be required to maintain a self-sustainable forest. Although jack pine suffered high mortality on this site, other species such as willow and poplar have thrived after direct planting on this deposit (Croser et al., 2000: Franklin et al., 2001). The relative tolerance of jack pine to salts and CT water in the controlled environment chamber suggests that this species could perform well in the field. In previous studies, planting failure has been linked to drought, wind, and heat (Cayford et al., 1967), and early growth and establishment is aided by light shading (Rudolf, 1958). The establishment of a nurse crop could reduce the effects of wind and provide some shade, thereby decreasing water deficit stress in planted jack pine seedlings. A clear trend toward increased survival of plants with increasing salt content of the pre-treatment solution was observed in the field experiment. This trend follows the results predicted by the hypothesis: that salt treatment had resulted in more salt tolerant seedlings. Results of the growth chamber experiment show that salt treatment did not convey any advantage to seedlings subsequently exposed to sub-lethal concentrations of salts alone. Conditions in the controlled environment chamber, however, were different from field conditions, with the main difference being water deficit stress. Amzallag (1997) was also not able to induce salt tolerance in sorghum plants under an artificial environment. Salt treatment therefore appears to have increased the frequency of traits conveying drought tolerance, rather than salt tolerance. Furthermore, the greatest survival rates were found in seedlings that had been treated with NaCl and CT water, where little mortality had occurred. These results suggest that salt treatment induced acclimation in the seedlings.

Acclimation to salt has been previously reported in *Sorghum bicolor* L. (Amzallag 1990). Pre-treatment of a longer duration may have been more effective in this study, as acclimation of *Sorghum bicolor* (L.) to NaCl was induced by pre-treatment with 150 mM NaCl for 20 days, but not by a pre-treatment of 14 days (Amzallag et al., 1990). Acclimation to salts has been demonstrated in sorghum pre-treated with NaCl and $CaCl_2$ (Richardson and McCree, 1985). Plants acclimated to salinity showed less reduction in photosynthetic rates than non-acclimated plants or plants acclimated to water stress (Zvi

and Federman, 1991), and greater survival rates and maintenance of turgor have been found in salt pre-treated plants under water deficit stress (Shalhevet, 1993). My results show that concentrations of Na in roots and shoots were greater in plants treated with NaCl than in those treated with isomolar Na_2SO_4. It is possible that higher solute contents of pre-treated plants allowed the uptake of water under lower soil water potentials. The acclimation of cotton to salinity was found to involve osmotic adjustment, primarily by the accumulation of Na and Cl (Plaut and Federman, 1991).

Injury, including needle necrosis and chlorosis, was observed in seedlings treated with salts and CT water in the growth room. Previous studies have shown conifers to be more affected by CT water than many broadleaf species, but less affected than raspberry and strawberry (Renault et al. 1998a). Injury resulting from NaCl treatment was greater than from isomolar Na_2SO_4, indicating that ion toxicity was more important than osmotic stress in salt-treated jack pine and in birch (*Betula papyrifera*) (Franklin et al., 2001). The reduced fresh weight of plants exposed twice to CT water in this growth room experiment suggests that some properties of CT, in addition to the major ions, may have a negative impact on plant growth, as similar weight reductions were not observed in treatments containing similar salt levels. In order to predict long-term effects of salts on jack pine growth, further work must investigate the mechanisms of ion toxicity, and the uptake and translocation rates of salt ions and mineral nutrients under saline conditions.

Controlled environment results suggest that jack pine is relatively tolerant of CT water, and so has potential as a reclamation species on CT-affected sites. Its establishment would be valuable ecologically as well as commercially. Once established, the young trees may aid in reducing salt levels in the upper layers of soil by increasing infiltration, and lowering of the water level (Tomar 1997). The poor survival rates seen in this study suggest that attention must be paid to microsite conditions when planting nursery-grown seedlings. I conclude that while pre-treatment of seedlings did not appear to select for salt tolerance, it may have induced acclimation. The use of saline treatment to increase survival rates of seedlings planted in arid conditions is worthy of further study.

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Chapter IV

Jack pine growth and elemental composition are affected by saline tailings water[1]

4.1 Introduction

Oil sands, a newly emerging energy source, are found throughout the world, with large deposits occurring in the United States, Venezuela, Colombia, and the Peace River, Cold Lake, and Fort McMurray regions of Alberta, Canada. The Athabasca oil sands reserves north of Fort McMurray, Alberta consist of Cretaceous age fluvial, estuarine, and marine sand deposits that are saturated with heavy crude oil (bitumen) and are overlain by shale (Jardine, 1974). These reserves are being developed aggressively, with two commercial mining operations (Syncrude Canada Ltd. and Suncor Energy Inc.) currently in production, and several other companies in pre-production stages. The sands are recovered by surface mining, and bitumen is extracted and upgraded to synthetic crude oil. Large volumes of solid and aqueous tailings remain after the separation of bitumen from the oil sand ore, and this will necessitate the eventual reclamation of land areas in excess of 50,000 ha. Much of the handled overburden and produced tailings materials will be more saline than the original soils, with some areas potentially providing challenging substrates in excess of 4dS/m conductivity. Current tailings management practices involve the containment of the tailings within settling basins, as well as a new technology that entails the re-mixing of the fine and coarse components along with gypsum ($CaSO_4.2H_2O$ at dosages of 750-1200 g/ton tailings) as a chemical coagulant to produce a non-segregating material known as consolidated or composite tailings (CT) (Matthews et al, 2000). This mixture is deposited as a slurry from which solids-free water is released. The goal is to produce a deposit that becomes trafficable, and reclaimable, within a shorter period than without this treatment. A large volume of the CT water will remain in this material and is expected to be expressed over a number

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of years as the deposit settles and de-waters further. These tailings and associated pore waters (CT water) are relatively saline, with Na and Cl being derived primarily from the ore, while most of the SO_4 comes from the gypsum treatment (MacKinnon et al., 2000).

Salinity may affect the growth of plants by altering water relations, by direct toxicity of ions, and by altering ionic balance resulting in nutrient deficiency. A reduction in water uptake may lead to a reduction in transpiration and photosynthesis, and therefore a reduction in growth. Nutrient imbalances may occur due to the inhibition or promotion of the uptake of nutritional elements under saline conditions (Alam, 1994), and by the relatively high pH of CT water. Injury to the plant tissues is also thought to occur due to the direct toxicity of ions (Greenway and Munns, 1980). Other components of CT water that may affect plant growth include B, Fl, Al and Sr (Renault et al., 2001).

Reclamation goals for the sites of current mining operations include the establishment of a stable and self-sustaining landscape with a productive capability at least equivalent to the pre-disturbed area. The establishment of a productive forest on much of the reclaimed lands will be required. Jack pine (*Pinus banksiana* Lamb.) is native to the Canadian boreal forest, with a range extending from Nova Scotia to northern British Columbia (Mirov, 1967). This pine is an early successional species commonly found on sandy, nutrient-poor sites, and may therefore be a suitable reclamation species for the oil sands mining areas. In previous studies, lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) showed a high degree of interspecific variability in response to CT water (Renault et al., 1998). One possible explanation for this difference among individuals is a difference in the uptake or translocation of salt ions or nutrients. Therefore, in the present study, I related seedling injury to the shoot tissue concentration of essential and non-essential elements.

The main objective of this study was to assess the tolerance of jack pine to CT water. This assessment was made on seedlings of two age classes; seven-month-old seedlings of planting age, which would be expected to be exposed to CT water when planted directly on reclamation sites, and younger one-month-old seedlings which may be affected by CT water shortly after germination in a future self-sustaining forest. The use of three seed sources reduced the possibility of testing an atypical population. The additional objective was to test the hypothesis that anticipated injury and growth

reduction in seedlings treated with CT water is related to the shoot tissue concentration of salt ions or nutritional elements. Identification of factors related to injury will allow for more efficient and potentially successful selection of suitable reclamation sites for jack pine placement.

4.2 Materials and Methods

4.2.1` Plant material

Jack pine seed was collected from two sites approximately 60 km north of Fort McMurray, Alberta (57°05.95'N 111°38.90'W and 56°56.06'N 111°31.95'W), and a third site near Smoky Lake, Alberta (54°06.88'N 112°10.38'W). Seeds were germinated in Petri dishes. Seeds, with emerged radicles approximately 10 mm long, were planted six days later in foam seedling blocks (Beaver Plastics, Edmonton, Alberta: 160 cavities/block, 60 ml vol/cavity) filled with quartz/feldspar sand (porosity of 28%) that had been washed with deionized water to eliminate silt and ions. Seedlings were grown in a controlled-environment chamber. Conditions during treatment and maintenance periods consisted of 70% relative humidity (RH), 24°/18° C (day/night) temperature and an 18-h photoperiod. A photosynthetically active radiation (PAR) of 300 to 350 $\mu mol\ m^{-2}\ s^{-1}$ was provided by a combination of cool white fluorescent and tungsten lamps. Seedlings were rotated randomly within the growth chamber once per week. Plants were watered daily with a nutrient solution containing 80 mg L^{-1} N, 60 mg L^{-1} P, 104 mg L^{-1} K, 100 mg L^{-1} Ca, 60 mg L^{-1} Mg, 79 mg L^{-1} S, 3 mg L^{-1} Fe, 0.40 mg L^{-1} Mn, 0.25 mg L^{-1} B, 0.14 mg L^{-1} Zn, 0.50 mg L^{-1} Cu, and 0.10 mg L^{-1} Mo, as recommended for local pine seedling production (Wood, 1995) (Appendix A). The containers with seedlings were flushed once a week with deionized water to prevent ion accumulation. For 7-month-old test material, budset was promoted at 10 weeks of age by reducing RH to 50%, and temperature to 14°/10° C (day/night) (Wood, 1995). At that time, watering was reduced to 2-day intervals, and nutrient solution was altered to contain 35 mg L^{-1} N,

80 mg L^{-1} P, and 50 mg L^{-1} Ca. Six weeks later, the photoperiod was reduced to 10 hours to induce dormancy for a period of 4 weeks prior to cold storage. Seedlings were stored for 8 weeks at 3° C in darkness, and watered every 10 days with deionized water.

4.2.2 CT water

In 1995, Syncrude Canada created a test deposit of consolidated tailings at its Mildred Lake site in Fort McMurray, Alberta. Water released from this deposit was collected in an adjacent lined pond, from which the CT water used in this experiment was collected in October of 1997. Water pH and conductivity were recorded, and samples were sent to Syncrude Research in Edmonton, Alberta for further analysis. Water samples filtered with 0.45-µm Millipore filters were analyzed for anion composition using an ion chromatograph (DI 300, Dionex Corporation, Sunnyvale, CA), and cations and trace metals were quantified using an optical emission spectrometer (ICP-OES) (Vista-PRO RL, Varian Analytical Instruments, Victoria, Australia). A Syncrude Canada Ltd. method, based on analysis of methylene chloride extracts of acidified samples (Jivraj et al., 1996), was used to determine naphthenic acid concentrations. The composition of this CT water is shown in Table 4.2.2.

4.2.3 Treatments

One-month-old seedlings were exposed to CT water in two steps. Seedling blocks were continuously sub-irrigated by placing them in trays containing deionized water (control) or a mixture of deionized water and CT water (1:1 by volume) to which nutrients had been added as described above. After two days, solutions were changed and the concentration of CT water increased from 50% to 100%. The level of solution in the watering trays was maintained by the daily addition of deionized water, which countered the concentration of salts and other elements by evaporation. After one week of treatment, the sand was flushed with deionized water to prevent surface salt accumulation caused by evaporation, and treatment solutions were replaced. One week later, seedlings were again flushed with distilled water, then watered daily with nutrient solution for the following 30 days. This final one-month period without CT water

Table 4.2.2. Chemical composition of consolidated tailings (CT) release water from the Syncrude's 1995 NST Field Test using gypsum treatment.

Component	Concentration
	mg L^{-1}
major cation	
Na^+	904
K^+	25.6
Mg^{2+}	23.8
Ca^{2+}	64.3
major anions	
F^-	2.1
Cl^-	490
SO_4^{2-}	1300
CO_3^{2-}/HCO_3^-	350
trace components	
B	3.5
Fe	0.238
Al	3.2
Sr	1.5
Mo	0.08
Mn	0.003
Ni	0.02
Cd, Cr, Cu, Se, Ti, Zn	<0.001
Naphthenic acids	43.8
other	
pH	9.05
SAR	24.4
Conductivity ($\mu S\ cm^{-1}$)	4390

SAR, sodium absorption ratio.

additions was required to accurately assess mortality, as preliminary studies showed high seedling mortality during this period in preliminary salt treatments.

The effects of CT water on seedlings of planting age were investigated by treating seven-month-old seedlings. These seedlings were dormant at the beginning of the treatment period. Plants were removed from the cold room, and immediately placed in nutrient solutions made in deionized water (control) or 50% CT water, in a controlled environment chamber at 70% RH, 24°/18° C (day/night) temperature and an 18-hour photoperiod. As in the previous experiment, CT water was increased to a concentration of 100% after two days. Seedlings were sub-irrigated on a cycle of 24 h, followed by 24 h without irrigation, with treatment solutions for a period of 10 weeks. Solutions were replaced weekly following flushing with deionized water, and the level of the solution in the watering trays was maintained by the daily addition of deionized water.

The experimental design for both one-month-old and seven-month-old studies was completely randomized, with the treatment unit of trays containing treatment solution being replicated four times, using 25 seedlings per replicate.

4.2.4 Measurements in one-month-old seedlings

Survival was monitored every second day throughout the 14-day treatment period, and every fourth day for the following one-month recovery period. Five plants per replicate were randomly selected and harvested for growth measurements and ion analysis. Plants were separated into roots and shoots, rinsed three times with deionized water, weighed, shoot length determined, then tissue was freeze-dried to determine dry weights. Chlorophyll a and b were determined spectrophotometrically in methanol extracts of freeze-dried needles, and calculated using MacKinney equations (Sestak et al., 1971). Shoot and root Na and K concentrations of freeze-dried tissue were determined by atomic absorption spectrophotometry (Perkins-Elmer 503) after digestion with sulfuric acid and hydrogen peroxide at 350° C (Richards, 1993).

4.2.5 Measurements in seven-month-old seedlings

Terminal bud flushing and survival were monitored every 4 days throughout the 10-week treatment period. At the end of this period, all shoots were harvested and washed three times with deionized water. Plants were weighed and stem length was determined, needles were separated into living (green) and necrotic (brown) portions, then the tissue was freeze-dried prior to dry weight measurements, and pigment and ion analysis. The percentage of needle necrosis was calculated based on the dry weight ratio of living and necrotic needles. Relative growth rate was determined from measurements taken at the beginning and end of the treatment period. Chlorophyll a and b were determined as described above, and total carotenoid content was measured spectrophotometrically in acetone extracts as described by Davies (1976).

For analyses of tissue mineral elements, five plants from the control group and fifteen plants from the CT treatment group were randomly selected from within a single seed source (57°05.95'N 111°38.90'W). The smaller sample size of control plants was found to be sufficient due to a low variance within the control group. Previously separated green needles, brown needles and stem from each shoot were recombined and homogenized. Mineral element analyses were performed by Envirotest Laboratory (Edmonton, Canada) using ICP-OES, as described by Renault et al. (1999). Tissue chloride concentrations were determined after extraction with 0.5 M HNO_3 for 30 min (Rieger and Litvin, 1998) using a chloride selective electrode (Accumet, Fisher Scientific, Edmonton, AB).

4.2.6 Data analysis

A general linear model (GLM) was used to test for interaction of seed source with treatment. No such interaction was found (Appendix B), and data from the three seed sources were combined for further analyses. Means of control and CT water treatment groups were compared by t-test. A correlation matrix was used to determine the factors most closely related to needle necrosis, shoot dry weight, and chlorophyll a content of 7-month-old seedlings.

4.3 Results

4.3.1 One-month-old seedlings

Survival of treated plants was greater than 95% over the two-week treatment period and subsequent one-month recovery period. All measured growth parameters and needle chlorophyll a concentration were significantly reduced by CT water treatment (Table 4.3.1.1). The relative reduction of growth was greater in roots (70% of control) than in shoots (80% of control). The reduction in needle chlorophyll b concentration in plants treated with CT water was not significant (Table 4.3.1.1). Tissue Na levels increased in shoots and roots of CT-treated plants by over 7, and 5-fold, respectively, above those of control plants (Table 4.3.1.2).

5.3.2 Seven-month-old seedlings

Survival was greater than 95% over the 10-week treatment period. Needle and stem dry weights and shoot growth rate were significantly reduced by CT water treatment (Table 4.3.2.1). Flushing of the terminal bud was delayed by three days in CT-treated plants, but this delay was not significant and analysis of covariance showed that it was not significantly related to the reduction in shoot weight. Needle necrosis varied greatly among seedlings of the CT treatment group, ranging from 1% to 29% with a mean of approximately 9% of the total needle weight, compared to 2% in control plants. Chlorophyll a and total carotenoid concentrations were significantly reduced by CT treatment, however chlorophyll b concentration was not affected. Assuming that the necrotic (brown) portion of the needles contained no pigments, pigment concentrations were re-calculated using the dry weight ratio of green to brown needles to reflect only the living needle pigment concentration. The significance of chlorophyll a and carotenoid reduction was maintained, indicating that the observed reduction in chlorophyll a and carotenoid content was not simply due to dilution of pigment concentrations by necrotic tissue in the sample.

Seedlings treated with CT water had significantly reduced levels of Fe, Mo, K and Ba in shoot tissue (Table 4.3.2.2). Shoot P, S, B, Cl, Na and Sr were significantly increased by treatment. Correlation of shoot elemental concentration with needle necrosis showed Na and Cl concentrations to be strongly related to needle necrosis ($r^2 = 0.65$ and $r^2 = 0.59$, respectively) (Fig. 4.3.2). No relationship was found between shoot elemental concentrations and shoot dry weight or needle pigment concentrations.

Table 4.3.1.1. Means and standard errors (in parentheses) for growth parameters and needle chlorophyll concentration of 30-day-old seedlings treated for 14 days with deionized water (control) or consolidated tailings (CT) water, followed by a 30-day recovery period. N = 100.

	Control	CT water	
Growth parameters			
Mean shoot dry weight (g)	0.084 (0.003)	0.067 (0.002)	*
Mean root dry weight (g)	0.039 (0.002)	0.027 (0.001)	*
Shoot length (mm)	56 (0.8)	51 (0.7)	*
Mean shoot elongation rate (mm day^{-1})	0.76 (0.01)	0.68 (0.01)	*
Chlorophyll concentration (mg kg^{-1} dry weight)			
Chlorophyll a	1370 (44)	1270 (28)	*
Chlorophyll b	260 (13)	250 (08)	

* indicates a significant difference from control at $\alpha = 0.05$ (one-tailed).

Table 4.3.1.2. Means and standard errors (in parentheses) of tissue Na and K concentration of 30-day-old seedlings treated for 14 days with deionized water (control) or consolidated tailings (CT) water, followed by a 30-day recovery period. N = 12.

Tissue	Element	Control	CT	
		(mg kg^{-1} dry wt.)		
Shoot	Na	280 (50)	2050 (300)	*
	K	15750 (610)	16290 (670)	*
Root	Na	740 (240)	3870 (380)	*
	K	21450 (660)	19760 (750)	

* indicates a significant difference from control at $\alpha = 0.05$ (two-tailed).

Table 4.3.2.1. Means and standard errors (in parentheses) of growth parameters and injury indicators (needle necrosis and pigment concentration) of 28-week-old seedlings treated for 10 weeks with deionized water (control) or consolidated tailings (CT) water. N = 16.

	Control		CT water		
Growth parameters					
Mean needle dry weight (g)	1.771	(0.083)	1.344	(0.066)	*
Mean stem dry weight (g)	0.189	(0.013)	0.138	(0.061)	*
Average flush time (days)	16.3	(1.1)	19.2	(1.5)	
Mean shoot elongation rate ($mm\ day^{-1}$)	0.38	(0.04)	0.22	(0.03)	*
Injury indicators					
Necrosis (% of total dry needle weight)	1.9	(0.5)	9.3	(2.0)	*
Chlorophyll a ($mg\ kg^{-1}$ dry weight)	1486	(96)	1078	(85)	*
Chlorophyll b ($mg\ kg^{-1}$ dry weight)	352	(30)	320	(28)	
Total carotenoids ($mg\ kg^{-1}$ dry weight)	92	(8)	56	(8)	*

* indicates a significant difference from control at $\alpha = 0.05$ (two-tailed).

Table 4.3.2.2. Means and standard errors (in parentheses) of elemental shoot tissue concentration of 28-week-old seedlings treated for 10 weeks with deionized water (control, N = 5) or consolidated tailings (CT) water (N = 15).

		Control		CT water		
		concentration (mg kg^{-1} dry wt.)				
Essential elements:	B	28.4	(1.72)	122.5	(8.31)	*
	Ca	3662	(355)	3177	(133)	
	Cl	868	(51.4)	6763	(827)	*
	Cu	3.40	(0.24)	3.60	(0.24)	
	Fe	32.60	(2.09)	26.13	(1.34)	*
	Mg	2798	(174)	2757	(97.9)	
	Mn	157	(8.04)	145	(8.14)	
	Mo	3.60	(0.68)	2.33	(0.25)	*
	P	1696	(128)	2243	(77.4)	*
	K	7018	(327)	4804	(327)	*
	S	1638	(108)	2908	(218)	*
	Zn	27.5	(6.13)	31.06	(3.82)	
Other elements:	Al	<10		<10		
	Ba	7.24	(1.09)	5.21	(.331)	*
	Cd	<0.5		<0.5		
	Cr	<0.5		<0.5		
	Na	<100		7295	(924)	*
	Ni	<2		<2		
	Pb	<5		<5		
	Sr	3.20	(0.58)	6.87	(0.46)	*
	Sn	13.20	(0.20)	12.79	(0.33)	

* indicates a significant difference from control at $\alpha = 0.05$ (two-tailed).

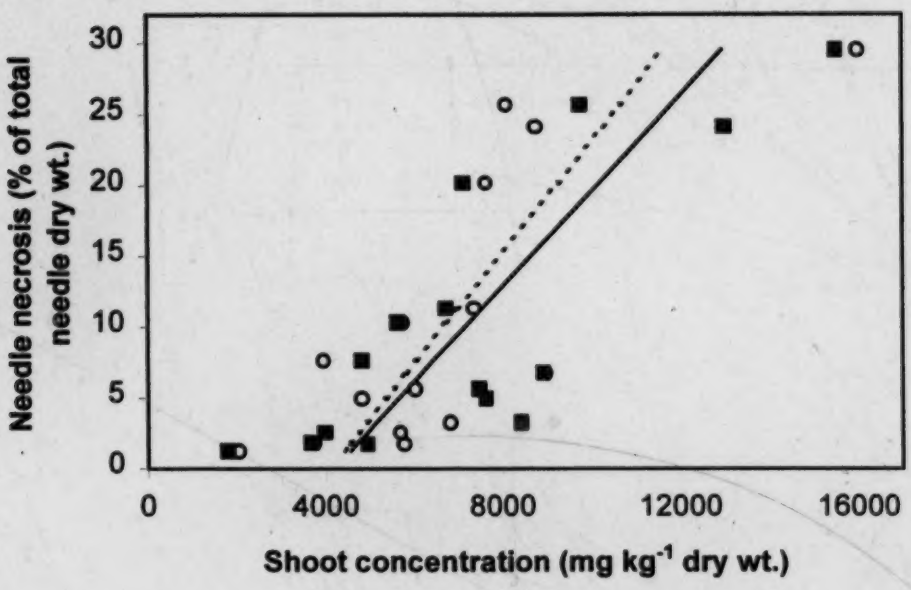

Fig. 4.3.2. Regression of needle necrosis with shoot Na and Cl concentrations, in 28-week-old jack pine seedlings treated for 10 weeks with consolidated tailings (CT) water. Solid squares represent Na concentrations, with a solid line indicating the regression line ($y = -14.02 + 0.00336x$, $r^2 = 0.65$). Open circles represent Cl concentrations, with a broken line indicating the regression line ($y = -15.99 + 0.00391x$, $r^2 = 0.59$).

4.4 Discussion

Although survival of jack pine seedlings treated with CT water was relatively high, both one-month-old and seven-month-old seedlings exhibited growth reduction and visible signs of injury. Under saline conditions, shoot growth is often found to be more affected than root growth, which may be due to altered plant hormonal relations (Poljakoff-Mayber and Lerner, 1994). In contrast, in the present study, one-month-old seedlings showed a greater growth reduction of roots than of shoots. Direct root damage could result from a high Na^+/Ca^{2+} ratio, which is thought to cause displacement of Ca from the plasma membrane (Cramer et al. 1985) and increased membrane permeability (Greenway and Munns, 1980). Root growth may also have been inhibited by factors other than salinity. One potential such factor is Al, which is known to affect root physiology (Cizkova, 1995), and has been found to accumulate in the roots of *Cornus stolonifera* Michx treated with CT water (Renault et al., 2001). Hovever, no accumulation of Al was found in roots or shoots of jack pine in this study. The observed reduction in shoot growth rate is a common response to salinity, and may result, in part, from osmotic stress that leads to a reduction in transpiration that subsequently limits photosynthesis. Growth reduction under saline conditions has also been attributed to a reduction in cell size due to decreased cell wall extensibility (Naqvi, 1994).

While reduced growth rates and dry weights are consistent with salinity stress, my results suggest that this growth reduction does not result from insufficient nutrient availability. Nutrient status is a concern due to the alkalinity of CT water, which reduces the solubility of N, P, Mn, Fe, and Mo. Although Fe, K and Mo concentrations were significantly reduced in CT treated plants, no relationship was found with plant dry weight. Altered ratios of K^+/Na^+ may affect growth, and this ratio is higher in more salt-tolerant provenances of *Pinus pinaster* (Aiton) (Saur et al., 1995). However, no relationship was found between the growth of pine seedlings and K^+/Na^+ ratios in this study. A slight increase in shoot K concentration of one-month-old seedlings occurred, in contrast with the significant decrease in seven-month-old seedlings after CT treatment. This difference could be due to differences in physiology or treatment duration of the two

age groups, as different effects predominate in short-term and long-term salt exposure (Greenway and Munns, 1980).

The effect of salinity on tissue Fe content varies widely among species (Grattan and Grieve, 1994), but my salt study (Chapter 3) showed no effect of NaCl or Na_2SO_4 on shoot Fe concentration in jack pine. The reduced shoot Fe concentration observed in this study could be the result of the high pH of CT water. Deficiencies in Fe and Mo are known to result in chlorosis (Salisbury and Ross, 1992), and this may have contributed to the observed chlorophyll reduction in treated jack pine, although no direct relationship was found. Similarly, nutrient status may have contributed to the observed reduction in carotenoid content, as total carotenoids have been found to decrease under Fe and K deficiency (Young and Britton, 1990). Tissue N content was not determined in this study, and could have been limiting, as high levels of Cl can inhibit NO_3 uptake (Grattan and Grieve, 1994).

Several elements were present in elevated levels in the shoot tissue of CT-treated seedlings. The most notable increases were in tissue Na and Cl. No relationship was found between needle dry weight and needle Na or Cl concentration, in contrast to the findings of Rogers et al. (1998), who found dry weight to be negatively correlated with shoot Na, Cl, and S concentration in *Medicago sativa* (L.) treated with NaCl and Na_2SO_4. Although not related to seedling growth, shoot Na and Cl concentrations were strongly associated with needle necrosis. Similar relationships between foliar NaCl and necrosis have been reported in *Pinus resinosa* (Aiton) (Sucoff et al., 1975) and *Pinus strobus* (L.) (Hall and Hofstra, 1972). Exposure to NaCl has been found to result in a loss of chlorophyll in many crop species including *Lycopersicon esculentum* (Mill.) (Khavari-Nejad and Mostofi, 1998) and *Medicago sativa* (L.) (Khavari-Nejad and Chaparzadeh, 1998). However, chlorosis is not a typical symptom of salt-affected woody plants (Bernstein, 1975), and no relationship was found between needle pigment content of CT-treated seedlings and Na or Cl concentrations. Na^+ and Cl^- can cause toxicity at high concentrations in plant tissues, but the mechanism of this toxicity is not fully understood. Martin and Koebner (1995) reported that Cl may be more toxic than Na, but that the effects of Na and Cl together were greater than the effects attributable to either ion alone in salt-treated *Triticum aestivum* (L.). Sodium concentrations of CT-treated jack pine

roots were nearly twice those in shoots of treated 30-day-old seedlings, indicating that jack pine possesses some mechanism to restrict Na translocation. The ability to limit Na accumulation by restricting uptake or translocation has also been reported for *Pinus radiata* (D. Don) (Myers et al., 1998).

I found significantly increased tissue S levels in the shoots of treated plants, reflecting the high SO_4 level present in CT water. Known effects of SO_4 include a disruption of N metabolism, and the promotion of toxic sulfoxide formation in leaves and roots of plants (Strogonov, 1973). An inhibition of cell division by sulfoxides may be a factor in the reduced growth of treated jack pine seedlings. In the current study, CT-treated plants also contained elevated levels of P. Salinity has been found to alter plant P status, with increases in tissue P observed for salt-treated *Pinus canariensis* (C. Sm.) (Tausz et al., 1998) and *Glycine max* ((L.) Merr.) (Grattan and Maas, 1988), and a decrease found in leaves of *Phaseolus vulgaris* (L.) (Carbonell-Barrachina et al., 1997).

Shoot tissue in CT-treated plants was found to contain significantly elevated levels of B and Sr. Shoot B in the control seedlings was well within the range of 11 to 32 mg kg^{-1} reported for pine species (Stone, 1990), while CT-treated seedlings had tissue B above the levels reported to result in tip necrosis in *Pinus resinosa* (Aiton) and *P. sylvestris* (L.). On sites with high soil B and salinity levels, *Carya illinoinensis* ((Wangenh.) K. Koch) showed a reduction in yield and a disruption of N and P translocation (Picchioni et al., 2000). Although elevated levels of B were found in the shoot tissue, these were not directly related to growth reduction or injury in jack pine. An accumulation of Sr in *Pisum sativum* (L.) has been found to result in growth reduction (Burstrom, 1983). Although Sr concentrations of CT-treated plants were significantly higher than controls, no correlation was found between tissue strontium and growth or injury. Small increases in tissue Sr, such as those found here, may have little impact on plant growth or injury due to possible compartmentalization within the shoot tissue, which has been demonstrated in *Larix decidua* (Mill.) (Gierth et al., 1998). The somewhat-elevated F^- in CT water may also have contributed to seedling injury and growth reduction. Sodium fluoride has been found to inhibit the growth of jack pine at concentrations as low as 1 mM (Zwiazek and Shay, 1988), but tissue fluoride was not determined in this study.

4.5 Summary

The alteration in nutrient status of plants treated with CT water appears to be related both to high pH and salinity. The observed reduction of shoot concentrations of several essential nutrients could potentially affect plant growth in the long term. Nutrient deficiency was not found to be directly related to plant growth or injury, and the factors responsible for growth reduction of CT-treated plants could not be identified in this study. The greatest concern is that of ion toxicity. The high Na and Cl levels in CT water were associated with needle necrosis, and salinity must, therefore, be considered in the development of reclamation plans for CT-affected areas. The loss of carotenoids is a potential problem, as one function of carotenoids is to protect the chloroplast from oxidative damage (Young and Britton, 1990). Resulting damage to the photosynthetic apparatus and the observed loss of chlorophyll a may reduce long-term growth. Future research with jack pine should examine the interactions of CT water chemistry with the physical properties of CT such as the high density, and investigate the potential of mycorrhizae to alleviate toxicity due to CT water chemistry.

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Chapter V

Growth and elemental composition of jack pine seedlings treated with NaCl and Na_2SO_4[1]

5.1 Introduction

Jack pine (*Pinus banksiana* Lamb.) is native to the Canadian boreal forest, with a range extending from Nova Scotia to northern British Columbia. Both naturally saline soils and salinity due to industry and development occur within this range, and these vary in the relative proportions of contributing ions. A large body of literature exists, concerning the effects of salinity on glycophytes, and this has been reviewed by Amzallag (1997), and Shannon et al. (1994). The majority of salinity studies have examined the effects of salt on agricultural species, and note decreased growth rates and leaf necrosis in response to salt treatments. While both osmotic effects and ion toxicity contribute to injury and growth reduction under saline conditions, their contributions may differ between species, and between tissue type or growth stage within a plant (Munns and Termaat 1986). Injury symptoms are often attributed to the toxicity of Na^+ and Cl^- ions, and reports of correlation between shoot Na^+ and Cl^- concentrations and growth or injury vary with species and cultivar. For instance, a positive relationship between shoot Na^+ and dry weight was found for chickpea (*Cicer arietinum*) (Lauter and Munns 1986), but not for rice (*Oryza sativa*) (Yeo and Flowers 1986). In red pine (*Pinus resinosa*), Cl^- was positively correlated with necrosis, but some trees with high tissue Cl^- and Na^+ did not show visible injury (Sucoff et al. 1975). Recent evidence suggests that the effects of salt on plant metabolism may be indirect; exposure of roots to salinity may alter hormonal concentrations in plant tissues (Amzallag 1997, Cheeseman 1988), and alter uptake of essential nutrients (Alam 1994). Salinity often results in reduced levels of shoot N, Ca^{2+}, and K^+, however, nutrient uptake is highly variable under saline conditions, particularly with respect to PO_4 and micronutrients, which may increase,

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decrease, or show no change, depending on the studied species and the type of salinity (Grattan and Grieve 1994).

Salinity type would be expected to have an impact on plant growth, where a direct ion toxicity or nutritional imbalance occurs (Lauchli and Epstein, 1990). Sodium is typically the most prevalent cation in saline soils, while Cl^- and SO_4^{2-} are the major anions. In sorghum (*Sorghum bicolor*) (Kahn et al. 1995) and in six tested wild potato (*Solanum*) species (Bilski et al. 1988), growth was more adversely affected by Na_2SO_4 than by equimolar NaCl, and blueberry (*Vaccinium corymbosum*) exhibited greater leaf damage when treated with Na_2SO_4 than with equimolar NaCl (Muralitharan et al. 1992). However, Cl^- ions are more readily absorbed than SO_4^{2-} ions by most plants (Bernstein 1975), and needle injury in ponderosa pine (*Pinus ponderosa*) was greater when treated with NaCl than with Na_2SO_4 (Spotts et al. 1972).

In this study I have investigated the effects of NaCl and Na_2SO_4 on the growth and nutritional balance of jack pine (*Pinus banksiana* Lamb.). I expected that nutrient imbalance, in addition to tissue salt levels, may explain both growth reductions and toxicity symptoms that were often attributed to the direct ionic toxicity of Na^+ or Cl-. I have used correlations to test the hypothesis that injury and growth reduction are related to shoot tissue concentration of salt ions, and nutritional elements.

5.2 Materials and Methods

5.2.1 Plant material

Jack pine (*Pinus banksiana* Lamb.) seeds were extracted from cones collected approximately 60 km north of Fort McMurray, Alberta (57°05.95N 111°38.90W). To ensure seedlings of equal age, seeds were germinated in Petri dishes, then seeds with emerged radicles approximately 10-mm long were planted. Planting media consisted of quartz/feldspar sand with a porosity of 28%, which had been washed with deionized water to eliminate silt and ions, then packed into cavities in foam seedling blocks (Beaver Plastics: 160/60 ml) to provide a total root volume of 60 ml per seedling. Seedlings were

grown in a controlled environment chamber at 70% relative humidity, 24°/18° C (day/night) temperature and 18-hour photoperiod. A fluence rate of 300 to 350 μmol m^{-2} s^{-1} was provided by a combination of cool white fluorescent (Sylvania) and tungsten lamps. Plants were watered daily with a nutrient solution (80 mg L^{-1} N, 60 mg L^{-1} P, 104 mg L^{-1} K, 100 mg L^{-1} Ca, 60 mg L^{-1} Mg, 79 mg L^{-1} S, 3 mg L^{-1} Fe, 0.40 mg L^{-1} Mn, 0.25 mg L^{-1} B, 0.14 mg L^{-1} Zn, 0.50 mg L^{-1} Cu, 0.10 mg L^{-1} Mo, pH 5.0 to 5.3) recommended for pine seedling production (Wood 1995). The sand was flushed weekly with deionized water to prevent ion accumulation, and seedlings were rotated randomly within the growth chamber. At 10 weeks of age, relative humidity was reduced to 50%, temperature to 14°/10° C, watering to every second day, and nutrient solution was altered (35 mg L^{-1} N, 80 mg L^{-1} P, 50 mg L^{-1} Ca) to promote budset. At 16 weeks of age, photoperiod was reduced to 10 hours to induce dormancy. From 20 to 28 weeks of age, dormant seedlings were stored at 3° C in darkness, and watered every 10 days with deionized water.

5.2.2 Treatments

Plants were removed from the cold room, and immediately placed in trays of nutrient solutions made in 20 mM NaCl, 20 mM Na_2SO_4, or deionized water (control), in a controlled environment chamber at 70% relative humidity, 24°/18° C (day/night) temperature and 18-hour photoperiod. Salt concentrations were increased by 20 mM per day to the final treatment concentration of 60 mM. Seedlings were bottom watered with treatment solutions for a period of 10 weeks, with the level of the solution in the watering trays maintained by the addition of deionized water. Once a week, plants were flushed by watering from the top with deionized water until the electrical conductivity of the pore water was less than 1.5 dS m^{-1}, and nutrient solution was replaced.

5.2.3 Measurements

Terminal bud flushing was monitored every 4 days. At the end of the treatment period, all shoots were weighed, measured, and washed briefly in deionized water. Needles were separated into green and brown portions in order to determine the

proportion of needle necrosis. As necrosis typically began at the needle tip and progressed basipedally, each needle was cut at the edge of the necrotic zone. The percentage of needle necrosis was calculated based on the dry weight of green and brown needles. Stem elongation rate was determined from measurements of stem height taken at the beginning and end of the treatment period. Tissue was freeze-dried prior to dry weight measurements, pigment and ion analyses. Chlorophyll a and b contents were determined spectrophotometrically in methanol extracts, and calculated using MacKinney equations (Sestak et al. 1971). Total carotenoid content was determined spectrophotometrically in acetone extracts as described by Davies (1976).

A total of 15 plants from each treatment group and 5 plants from the control group were randomly selected for tissue analysis. To obtain the quantity of tissue required for elemental analysis, all needle and stem portions from a single shoot were combined and homogenized. Elemental analysis was performed by Envirotest Laboratory (Edmonton, Canada) using ICP-OES as described by Renault et al (1999). Tissue Cl concentrations were determined after extraction with 0.5 M HNO_3 for 30 min (Rieger and Litvin 1998) using a Cl selective electrode (Accumet, Fisher Scientific, Edmonton, AB). Shoot total N concentrations of 5 plants per treatment group were determined by atomic absorption spectrophotometry (Perkins-Elmer 503) after digestion with sulfuric acid and hydrogen peroxide at 350° C (Richards 1993).

Data were analyzed using a general linear model (GLM) (Appendix B). Means were compared by Tukeys' test. Within each treatment group, a correlation matrix was used to determine the strength of the relationships between significant growth and injury factors, and shoot concentrations of salt ions and nutritional elements. Correlations were considered significant at $p \leq 0.05$.

5.3 Results

Survival in all treatments over the 10-week treatment period was greater than 95%. Shoot dry weight was reduced by 36% in 60 mM Na_2SO_4-treated seedlings, and by 40% in 60 mM NaCl-treated seedlings (Table 1). The relative water content of green needles

and stem was slightly, but significantly, elevated in seedlings treated with NaCl in comparison to controls, and flushing of the terminal bud was significantly delayed (Table 1). The percentage of seedlings exhibiting terminal bud flush decreased slightly, from 98.4% in the control seedlings to 91.9% and 90.2% in Na_2SO_4 and NaCl treatments, respectively. Seedling growth rate was significantly reduced by both salt treatments, with the growth rate in Na_2SO_4 being less than half that of the control seedlings (Table 1). Needle injury first appeared after five weeks of treatment, and was characterized by a browning and drying of the needle tip that progressed slowly toward the base of the needle. The demarcation between green and necrotic tissue was abrupt. While a small but significant amount of needle necrosis (8%) occurred in Na_2SO_4-treated seedlings, NaCl treatment resulted in a significantly greater necrosis of approximately 21% of the needle dry weight (Table 1). Chlorophyll a and total carotenoid content were reduced in NaCl-treated plants.

Elemental analysis of shoots showed seedlings treated with Na_2SO_4 to have significantly reduced tissue concentrations of Ca and K, while levels of P and S were significantly increased (Table 2). Na concentrations were more than 130 times that of controls. Shoot Fe concentrations did not differ from controls in either treatment group (Table 2). Shoot tissue of seedlings treated with NaCl had significantly elevated levels of K, Mg, Mn and P. The levels of Cl were more than 30 times that of control seedlings, and Na concentrations were 106 times that of controls (Table 2). Tissue Na was positively correlated with needle necrosis in NaCl-treated seedlings (Fig. 1a). No such correlation was found in seedlings treated with Na_2SO_4 (Fig. 1b). Although there was a significant correlation between tissue Na and Cl in NaCl-treated plants ($r^2 = 0.442$), tissue Cl was not found to be significantly correlated with needle necrosis in NaCl treated seedlings ($r^2 = 0.24$). No relationships were found between the tissue concentration of nutritional elements and needle necrosis (Table 2), or between elemental composition and growth parameters or pigment content.

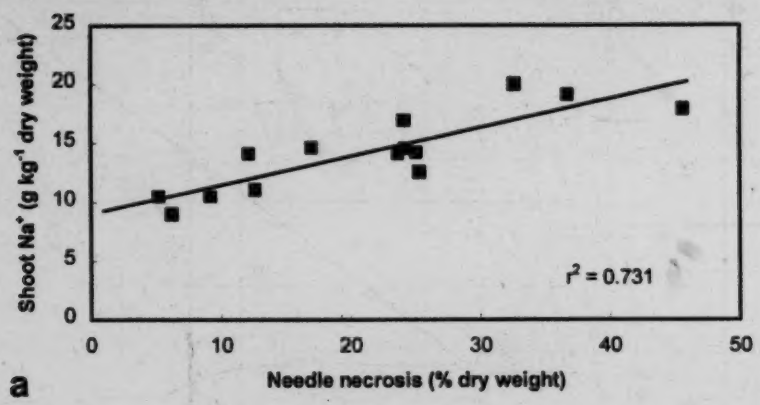
Table 5.3.1. Growth measurements, needle necrosis and pigment content of jack pine seedlings treated for 10 weeks, beginning at 28 weeks of age, with salt solution (n = 20). Standard errors are shown in parentheses. Different letters indicate significant differences between treatments at $\alpha = 0.05$.

	Control	60 mM Na_2SO_4	60 mM NaCl
Shoot dry weight (g)	1.35 (0.18) a	1.09 (0.06) b	0.92 (0.05) b
Water content of green needles (% fresh wt.)	0.631 (0.008) a	0.645 (0.017) a	0.723 (0.004) b
Water content of stem (% fresh wt.)	0.669 (0.013)	0.666 (0.029)	0.725 (0.010)
Average number of days to bud flush	16.27 (1.14) a	18.78 (1.59) ab	23.67 (1.47) b
Stem elongation rate (mm day^{-1})	0.383 (0.041) a	0.150 (0.014) b	0.203 (0.041) b
Needle necrosis (% of needle dry weight)	1.89 (0.49) a	7.89 (1.28) b	21.44 (2.43) c
Chlorophyll a (mg g^{-1} dry weight)	1.73 (0.14) a	1.47 (0.09) ab	1.36 (0.17) b
Chlorophyll b (mg g^{-1} dry weight)	0.436 (0.037)	0.460 (0.031)	0.363 (0.046)
Total carotenoids (mg g^{-1} dry weight)	0.092 (0.008) a	0.081 (0.007) ab	0.047 (0.012) b

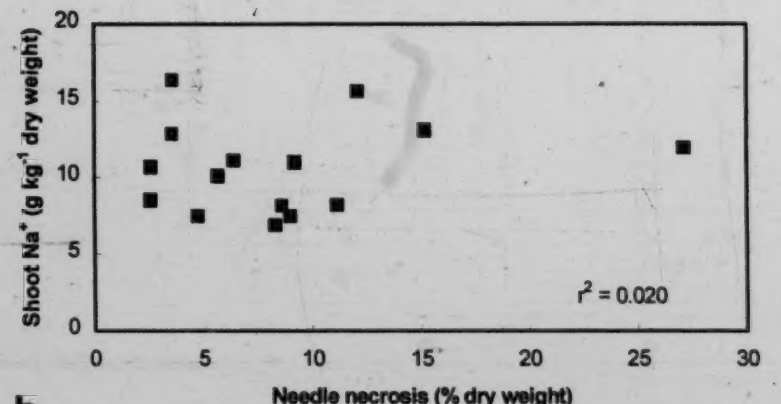
Table 5.3.2. Elemental content (mg g^{-1} dry weight) and Pearson correlation coefficients of tissue elemental composition with needle necrosis (r) of jack pine shoots treated for 10 weeks, beginning at 28 weeks of age, with salt solution. Standard errors are shown in parentheses. n = 5 (control) or n = 15 (salt treatments). Different letters indicate a significant difference between treatments at $\alpha = 0.05$.

	Control concentration ($mg\ kg^{-1}$)	60 mM Na_2SO_4 concentration ($mg\ kg^{-1}$)	r	60 mM NaCl concentration ($mg\ kg^{-1}$)	r
Ca	3.662 (0.355) a	2.729 (0.098) b	0.154	3.998 (0.142) a	-0.320
Cl	0.807 (0.051) a	1.029 (0.184) a	0.307	26.523 (2.063) b	0.494
Cu	0.0034 (0.0002)a	0.0043 (0.0002) ab	0.038	0.0049 (0.0002)b	-0.152
Fe	0.0326 (0.0021)	0.0291 (0.0013)	0.209	0.0341 (0.0010)	-0.194
K	7.018 (0.327) a	4.970 (0.427) b	-0.114	9.167 (0.469) c	0.095
Mg	2.798 (0.174) a	2.629 (0.089) a	0.302	3.428 (0.141) b	-0.041
Mn	0.157 (0.008) a	0.193 (0.011) ab	-0.106	0.238 (0.011) b	0.077
N	938 (44) c	1354 (54) b	0.405	1530 (36) a	0.328
Na	0.100 (0.010) a	13.859 (0.759) b	0.142	10.612 (0.908) b	0.855 *
P	1.696 (0.128) a	2.882 (0.122) b	-0.159	2.503 (0.089) b	0.229
S	1.638 (0.108) a	7.169 (0.381) b	0.095	1.855 (0.086) a	0.243
Zn	0.0275 (0.0061)	0.0379 (0.0033)	0.050	0.0340 (0.0019)	0.222

* indicates a significant correlation at $\alpha = 0.05$



a



b

Fig. 5.3.1. Relationship between needle necrosis and Na content of shoot tissue, in jack pine seedlings treated at 28 weeks of age for 10 weeks with (a) 60 mM NaCl or (b) 60 mM Na_2SO_4.

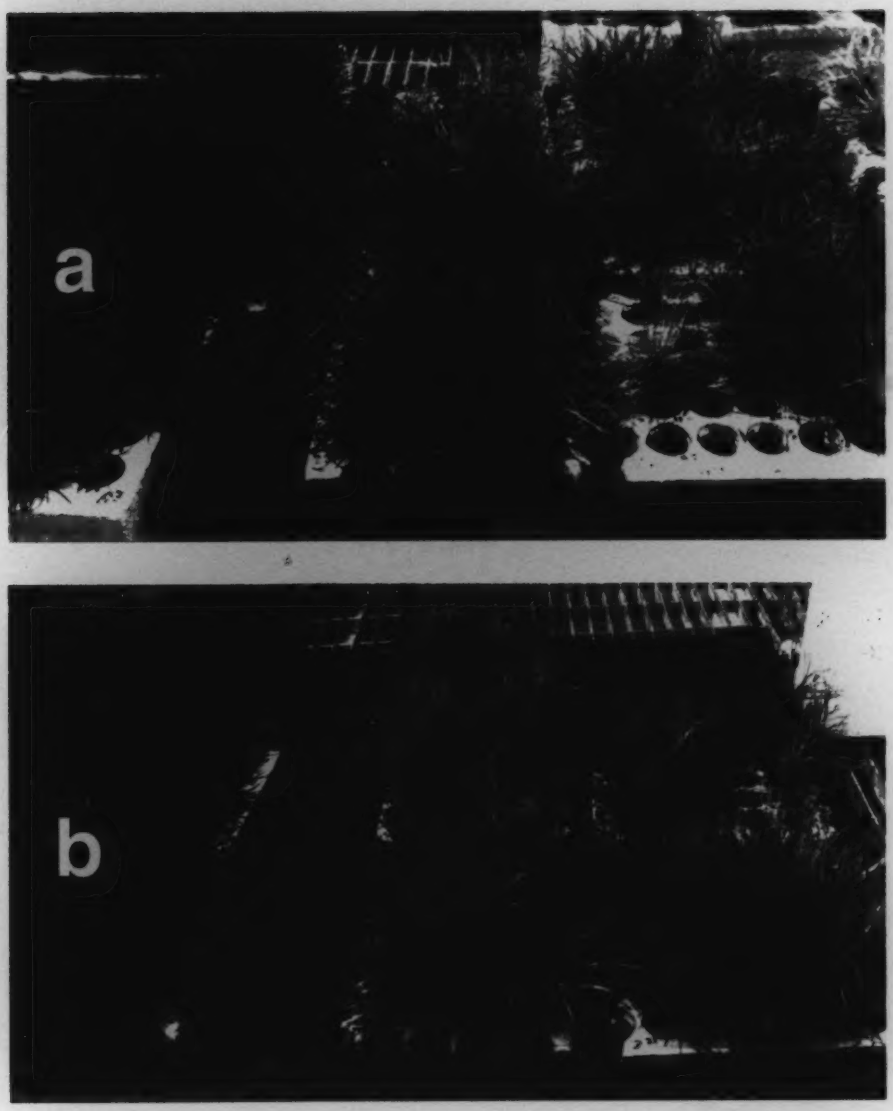


Fig. 5.3.2. Photographs of seedlings at the end of 10 weeks of treatment with 60 mM Na_2SO_4 (a) or 60 mM NaCl (b). Control seedlings are shown on the left-hand side of each photo.

5.4 Discussion

Jack pine seedlings were generally more adversely affected by NaCl treatment than by Na_2SO_4 (Table 5.3.1). This concurs with previous findings that woody plants tend to be more sensitive to excess Cl than to high levels of Na (Shannon et al. 1994). In Na_2SO_4-treated plants, stem elongation rate was affected to a greater extent than was shoot fresh weight, with the reverse being found in the NaCl treatment (Table 5.3.1). This difference in growth resulted in shorter and heavier seedlings in the Na_2SO_4 treatment than in the NaCl treatment, possibly due to differing rates of cell elongation and cell division, as SO_4 has been found more inhibiting to the former, and Cl, to the latter (Strogonov 1974). Osmotic potential of the Na_2SO_4 treatment was lower than that of the NaCl treatment, which could result in larger decreases in turgor, and thus cell elongation. However, salinity could also affect cell elongation indirectly, through changes in cell wall extensibility, hydraultic conductiviey or yield threshold, although the roles of these factors have not yet been clearly demonstrated (Cramer and Bowman, 1994).

A reduction in shoot dry weight of salt-treated seedlings could, in part, be explained by stomatal closure caused by the lowered substrate water potential. However, this weight reduction was greater in NaCl-treated plants, although the osmotic potential of the Na_2SO_4 treatment solution was much lower than that of the NaCl solution. Therefore, altered water relations alone cannot explain the reduced weights of seedlings treated with NaCl. Sands and Clarke (1977) who compared injury in radiata pine (*Pinus radiata*) treated with salts or PEG (polyethylene glycol) 4000 also concluded that salt-induced drought stress alone was not the cause of injury or mortality. Shoot growth in these plants may have been hindered by ion toxicity or nutrient deficiency.

A significant amount of needle necrosis occurred only in the NaCl-treated seedlings (Table 5.3.1), but necrosis was not related to levels of Cl in shoot tissue. A correlation has been found between Cl content of needles and needle browning (Sucoff et al. 1975, Spotts et al. 1972); however, tissue Cl did not completely explain the cause of needle injury as some trees with very little visible injury had a high needle Cl content (Sucoff et al. 1975). A greater degree of needle injury in NaCl- than in Na_2SO_4-treated

seedlings was previously observed in ponderosa pine (Spotts et al. 1972), where necrosis was observed in 3-year-old seedlings after 2 months of treatment. In contrast to my findings, Cl was the factor with the greatest correlation to needle necrosis in ponderosa pine, while Na was not found to be correlated with necrosis (Spotts et al. 1972). It was observed however, that necrosis was more severe in Cl-treated plants as the amount of Na in the treatment solution increased. Correlations in the above study were made across treatments, while my data have been analyzed within treatments (Table 5.3.2). Analyzed across treatments, my results are similar to those obtained by Spotts et al. (1972).

A slightly higher concentration of Na was found in the shoots of Na_2SO_4-treated plants than in the shoots of NaCl-treated plants (Table 5.3.2), although the former treatment solution had twice the Na concentration of the latter. These Na concentrations did not appear to result in injury to the needles of plants treated with Na_2SO_4. In plants treated with NaCl, it is unclear whether high Na levels were the cause of needle injury, or whether Na simply accumulated in physiologically senescent tissue. By testing the effects of a wide range of salts on the growth of wheat (*Triticum vulgaris*), Martin and Koebner (1995) observed that the effects of NaCl on growth and photosynthetic capacity were greater than the additive calculated contributions of Na^+ and Cl^- ions. They proposed that this synergistic effect may be due to increased membrane permeability resulting from Ca^{2+} displacement from the plasma membrane, and leading to accumulation of Cl^- in the cytosol. My results, however, do not support this hypothesis as the mechanism of injury in NaCl-treated jack pine seedlings.

A high Na^+/Ca^{2+} ratio is thought to cause displacement of Ca^{2+} from the plasma membrane resulting in decreased Na^+/K^+ selectivity (Cramer et al. 1985). This is possibly the case in my Na_2SO_4-treated seedlings, as shoot tissue had reduced levels of Ca^+ and K^+, and very high levels of Na^+ (Table 5.3.2), reflecting the high Na^+/Ca^+ and Na^+/K^+ ratios of the treatment solution. However, seedlings in this treatment showed little needle injury, and no correlation was found between injury and tissue Na. NaCl-treated plants, however, showed a slight increase in Ca, and a significant increase in K^+ and other cations (Na^+, Mg^{2+}, Mn^{2+}, Cu^{2+}). My data suggest that the presence of Cl may impede the ability of the plant to restrict the movement of Na^+ and other cations.

Chloride has been found to induce changes in plasma membrane permeability resulting from altered lipid composition (Kuiper 1968).

The uptake of mineral nutrients under saline conditions varies greatly between species (Grattan and Grieve 1994). Needles of radiata pine exposed to NaCl were found to have increased levels of K and PO_4, but needle Ca and Mg did not differ greatly from controls (Sands and Clarke 1977). In contrast, ponderosa pine showed increased Ca and Mg when treated with NaCl, but not when treated with Na_2SO_4 (Spotts et al. 1972), similar to the results of this study. Although carotenoids have been found to decrease under iron and K deficiency (Young and Britton, 1990), carotenoid content of jack pine was reduced only in NaCl-treated plants, which had increased tissue levels of these elements. Although not statistically significant, reduced levels of chlorophylls a and b were also found in NaCl-treated plants, but were not related to shoot nutrient status.

In jack pine, injury did not appear to be due to nutrient deficiency, as tissue levels of mineral nutrients were higher in NaCl treated seedlings than in control seedlings. While high levels of Cl are often found to inhibit NO_3^- uptake (Grattan and Grieve, 1994), the N concentration of maritime pine (*Pinus pinaster* Ait.) needles increased in plants treated with NaCl (Saur et al., 1995), similar to the results of this study. It is also possible that injury and growth reduction resulted from an excess of mineral nutrients in the tissues, or from changes in the availability of nutrients within the cell due to ion imbalances. Phosphorous, in particular, has been shown to have a synergistic effect with Cl in the injury of soybean (Grattan and Maas 1988). Although no elements other than Na showed a correlation with injury in this study, it is possible that high levels of PO_4 or other elements in the tissue contributed to injury or growth reduction of jack pine seedlings.

In summary, I found that growth and injury of jack pine seedlings were more affected by NaCl than by equimolar Na_2SO_4, and that injury was related to tissue Na only in the former treatment. Reduced levels of mineral nutrients were found in shoots treated with Na_2SO_4, but not NaCl, and shoot nutrient content was not related to growth or injury in either treatment. I propose that in jack pine, the presence of Cl results in an increase in the translocation of Na and other cations to the shoot. Accumulation of these elements, particularly Na, in the needle tissue may then result in injury. Further study is necessary

to determine the roles of Na and Cl in needle injury. These roles may be clarified by the assessment of tissue ion content prior to the appearance of necrosis (Greenway and Munns 1980), and are worthy of further investigation.

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Chapter VI

Tissue sodium and membrane injury are related to tissue Cl in jack pine treated with NaCl and Na_2SO_4

6.1 Introduction

Jack pine (*Pinus banksiana* Lamb.) occupies a vast area in North America reaching from northern British Columbia to Nova Scotia, and southward into the Great Lakes region. Some saline soils occur within this range, as do increasing areas of salinity originating from road salt application, brine spills, and mining activity. Extraction of bitumen from the Athabasca oil sands in northern Alberta creates large areas of saline tailings that must be reclaimed. The main salts that are present in these tailings are NaCl and Na_2SO_4, in varying proportions. It is, therefore, necessary to understand how these salts affect plants, in order to predict plant growth response on a given reclamation site and develop an appropriate reclamation strategy. An understanding of the rate of uptake of salts and nutritional elements and their distribution within the plant is useful in predicting the long-term effects of salinity on jack pine growth and health.

The stress imposed by salinity is complex, and may be both direct, through osmotic effects and ion toxicity (Jacoby, 1994), and indirect, through nutrient deficiency (Grattan and Grieve, 1994) and alteration of hormonal balances (Poljakoff-Mayber and Lerner, 1994). The different effects of NaCl and Na_2SO_4 vary greatly with the species in question. Growth was more reduced by Na_2SO_4 than by isomolar NaCl in sorghum (*Sorghum bicolor* L.) (Khan et al., 1995) and in dogwood (*Cornus stolonifera* Michx) (Renault et al., 2001), and more necrosis observed in highbush blueberry (*Vaccinium corymbosum* L.) treated with Na_2SO_4 than isomolar NaCl (Muralitharan et al., 1992). However, interpretation of results comparing these two salts is complicated by the reality that Na_2SO_4 imposes a greater osmotic stress, and contains twice the Na as isomolar NaCl. Similar difficulties occur when working with iso-osmotic solutions, or milliequivalent Na solutions. My previous studies on jack pine have shown greater needle necrosis in seedlings treated with 60 mM NaCl than with 60 mM Na_2SO_4.

Because the NaCl solution contained less Na and had a lower osmotic potential than the Na_2SO_4 solution, injury cannot be explained by either Na toxicity or osmotic stress and must, therefore, be attributed to the presence of the Cl^- ion.

Many woody species appear to be sensitive to Cl (Shannon, 1994). However, the relationship between tissue Cl concentrations and injury is not clear, and is complicated by the close association of Na uptake with that of Cl. Some NaCl tolerant tree species such as red and white oak (*Quercus rubra* L., *Q. alba* L.) had low accumulation of Cl in tissue, while other species such as paper birch (*Betula papyrifera* Marshall) appear to be able to tolerate high tissue Cl concentrations (Shortle and Rich, 1970). Within a species, needle Cl and Na concentrations were related to necrosis of red pine (*Pinus resinosa* Aiton) but trees with high needle Cl content varied widely in their level of injury (Sucoff et al., 1975). Such a variation in response may be due to differences in the ability of plants to compartmentalize ions within the tissue (Hasegawa et al., 1986). When present at high concentrations within the plant tissue, Cl may inhibit enzyme action and protein synthesis (Greenway and Munns, 1980). Another proposed mechanism of Cl toxicity is the induction of membrane leakage (Bernstein, 1975). Chloride has been found to induce changes in plasma membrane permeability resulting from altered lipid composition (Kuiper 1968). This increase in permeability may limit the ability of the plant to regulate the entry of other solutes into the cell, which may, in turn, cause additional injury. Evidence for increased Na uptake or translocation was seen in dogwood (Renault et al., 2001), highbush blueberry (Muralitharan et al., 1992) and sorghum (Khan et al., 1995), where plants grown in NaCl had higher tissue concentrations of Na than did plants grown in Na_2SO_4 solutions containing the same level of Na. Treatment of corn (*Zea mays* L.) with $NaCl + CaCl_2$ has been found to increase the permeability of the membrane to PO_4 (Roberts et al., 1984).

Seedlings were grown in sand culture, and treated with different concentrations of NaCl or Na_2SO_4 solutions to compare the effects of isomolar salts, and milliequivalent Na levels. Electrolyte leakage, total plant ion uptake, root-to-shoot transport, and tissue concentrations of Na, Cl, Ca, K, P, and S were determined from plants examined after different treatment durations to test the hypothesis that the presence of Cl results in increased membrane permeability and that this increase corresponds to an increased

uptake and translocation of Na. The results show increased rates of Na uptake and electrolyte leakage in the presence of Cl.

6.2 Materials and Methods

6.2.1 Plant material and treatments

Dormant, one-year-old jack pine (*Pinus banksiana* Lamb.) seedlings were obtained from Pine Ridge Forest Nursery, in Smoky Lake, Alberta. The roots, which had been growing in a peat/perlite mix, were washed thoroughly in deionized water. Seedlings were then planted into quartz/feldspar sand that had been washed with deionized water, in the cavities of "Spencer-Lemaire rootrainers" (350 ml volume, Stuewe and Sons, Inc., Corvallis). Plants were placed in a controlled environment chamber at 70% relative humidity, 24°/18° C (day/night) temperature and 18-hour photoperiod. Photosynthetically active radiation of 250 to 300 μmol m^{-2} s^{-1} was provided by cool white fluorescent lights (Sylvania). Plants were watered 3 times a week with a nutrient solution (80 mg L^{-1} N, 60 mg L^{-1} P, 104 mg L^{-1} K, 100 mg L^{-1} Ca, 60 mg L^{-1} Mg, 79 mg L^{-1} S, 3 mg L^{-1} Fe, 0.40 mg L^{-1} Mn, 0.25 mg L^{-1} B, 0.14 mg L^{-1} Zn, 0.50 mg L^{-1} Cu, 0.10 mg L^{-1} Mo, pH 5.0 to 5.3) recommended for pine seedling production (Wood 1995).

Seedlings were maintained under the above conditions for 1 week to allow for recovery from transplanting. Treatments were then applied by placing seedling containers in trays filled with nutrient solution (control), or nutrient solution containing 60 mM NaCl, 60 mM Na_2SO_4, 120 mM NaCl, or 60 mM NaCl + 30 mM Na_2SO_4. Plants were alternated between trays of treatment solution and the bench every 24 hours to facilitate free drainage from the bottom of the containers. Three replicate groups of seedlings and trays of treatment solution were used in randomized complete block design.

6.2.2 Measurements

Six plants were harvested at the beginning of the experiment to serve as baseline data. At 1 week, 3 weeks, and 5 weeks after the start of treatment, six plants per

treatment (two plants per replicate) were harvested. Harvested plants were rinsed in 3 changes of deionized water (3 x 30 seconds), and divided into roots, stem, brown (necrotic) needle portions, green (living) needle portions, and needles of the current years growth (if present). Fresh weights were recorded for all tissues.

Electrolyte leakage was determined for stems, green needles of the previous season's growth, and new needles using a procedure modified from that described by Zwiazek and Shay (1988). Weighed amounts of needle or stem tissue were cut into 1 cm segments and washed for 1 hour in deionized water on an orbital shaker at 50 rpm. The water was replaced, and the electrical conductivity of the solution was measured after 5 hours (EC_I) using a portable conductivity meter (HI 8033, Hanna Instruments, RI). The tissue in solution was then placed in a pressure cooker at 251° C for 15 minutes, frozen at -85° C overnight, then warmed to 25° C before measuring tissue total electrolytes (electrical conductivity = EC_T). Electrolyte leakage was calculated as the percentage of total electrolytes in solution after 5 hours ($EC_I / EC_T * 100$). Tissue not used for measurement of electrolyte leakage was freeze-dried, weighed, ground in liquid nitrogen to a size that would pass through a 2-mm sieve, and analyzed for ion content. The percentage of needle necrosis was calculated based on the dry weight ratio of living and necrotic needles.

6.2.3 Ion concentration, uptake, and translocation

Cation content was quantified using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Vista-PRO RL, Varian Analytical Instruments, Victoria, Australia) after microwave digestion of samples in nitric acid and filtration with 0.45 μm Millipore filters (Csikkel-Szolnoki, 1996). Anion content was determined by ion chromatography (DI 300, Dionex Corporation, Sunnyvale, CA) on a filtered water sample obtained by combining two successive 1-hour extractions of the sample with boiling water. It was necessary in some treatments to combine new needles or necrotic needles from all plants within a replicate to obtain sufficient quantities of tissue for cation and anion determination. For control plants, it was necessary to combine necrotic tissue

from all plants within the treatment. Rates of ion uptake and translocation between root and shoot were calculated for each time interval, as described by Pitman (1988):

$$J_{Tot} = [(M_{Tot2} - M_{Tot1})/(T_2-T_1)] * (1/W_R)$$

Where J_{Tot} is the ion flux into the plant, M_{Tot1} and M_{Tot2} are total plant ion content at times T_1 and T_2, and W_R is the average root weight.

$$J_S = [(M_{S2} - M_{S1})/(T_2-T_1)] * (1/W_R)$$

Where J_S is the root-to-shoot transport rate, M_{S1} and M_{S2} are shoot ion content at times T_1 and T_2, and W_R is the average root weight.

6.2.4 Data analysis

Data were analyzed using a general linear model (GLM). Means of treatments were compared by Duncans' multiple range test at each harvest date. Uptake and transport rates of Ca and Mg did not differ significantly between time intervals, and the data was combined for further analysis. A correlation matrix was used to determine the strength of the relationships between tissue Cl and Na concentrations, needle necrosis, and electrolyte leakage of stems and green needles. These data were analyzed across all treatments, and separate analyses were made on treatments containing 60 mM Cl, and on 60 mM Na_2SO_4.

6.3 Results

6.3.1 Growth

Dry weights of roots, stems, and current year needles were similarly reduced by all salt treatments (Fig. 6.3.1). Needles from the previous year's growth were maintained in control plants, but suffered necrosis in salt treated plants as seen by the low weight of

"old" needles and large weights of necrotic needles (Fig 6.3.1). The amount of needle necrosis did not differ between treatments containing Cl (60 mM NaCl, 120 mM NaCl, and 60 mM NaCl + 30 mM Na_2SO_4), but was significantly lower in plants treated with 60 mM Na_2SO_4.

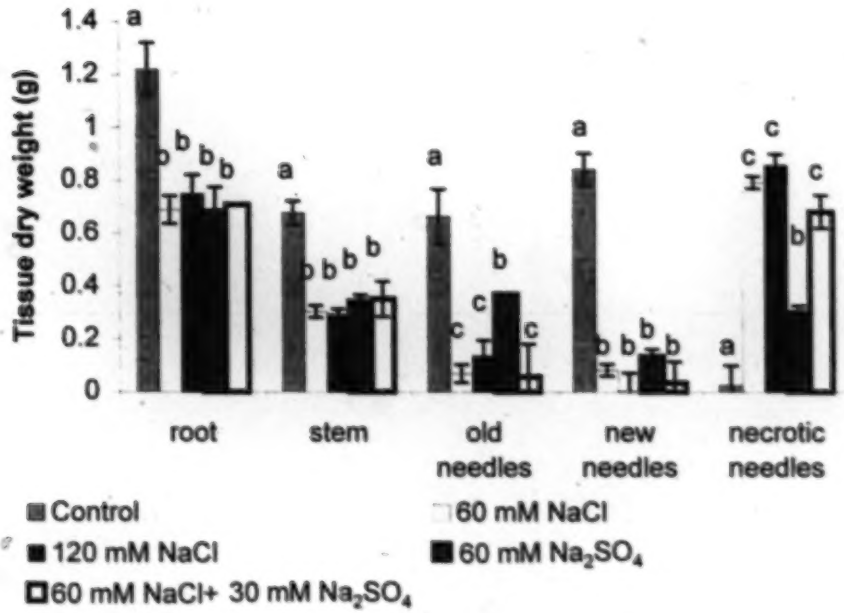

Fig. 6.3.1. Tissue dry weights of root, stem, living needles of the previous years growth (old), living needles of the current years growth (new), and necrotic needles of plants treated for 5 weeks with control or salt solutions. Bars represent ± SE. Different letters indicate significant differences between treatments at $\alpha = 0.05$.

6.3.2 Tissue ion concentrations

Sodium concentrations in roots (Fig. 6.3.2) increased in a linear manner for the first 3 weeks, then increased only slightly over the next 2 weeks. This pattern was similar for all salt treatments. Sodium concentrations in old needles increased little during the first week, and showed a significant increase over the following four weeks. Sodium concentrations after 5 weeks did not differ significantly between treatments containing Cl, but were significantly higher than those in the 60 mM Na_2SO_4 treatment. In stems,

Na concentrations were similar to those of old needles in all treatments, while Na concentrations in necrotic needles were slightly lower than in old needles. New needles of plants treated with 60 mM NaCl had Na concentrations approximately 20% greater than in old needles after 5 weeks of treatment.

Cl concentrations in roots increased over time (Fig. 6.3.3), with the highest concentrations of around 17000 mg kg^{-1} dry wt. While root Cl concentrations were about 20% higher than root Na in 60 mM and 120 mM NaCl treated plants, Cl concentrations in plants treated with mixed salt were 56% of root Na concentrations. After 5 weeks of treatment, Cl concentrations of roots were significantly different between all 3 chloride treatments, with the lowest concentration found in plants treated with 60 mM Cl in the mixed salt (7406 mg kg^{-1} dry wt.), the highest in those treated with 120 mM NaCl (16700 mg kg^{-1} dry wt.), and plants treated with 60 mM NaCl showing intermediate concentrations (12100 mg kg^{-1} dry wt.). In needles and stems of Cl-treated plants, Cl concentrations patterns of Cl accumulation were similar to those of Na (Fig. 6.3.3), although concentrations tended to be much higher, reaching a maximum of 62000 mg kg^{-1} dry wt. in new needles of plants treated with 60 mM NaCl + 30 mM Na_2SO_4. New needle growth in plants treated with 120 mM NaCl was insufficient for analysis. After 5 weeks of treatment, Cl concentrations of old needles differed between treatment Cl concentrations (0, 60 mM and 120 mM), but did not differ between the two 60 mM Cl treatments (Fig. 6.3.3).

Tissue concentrations of SO_4 in plants treated with mixed salt (containing 30 mM SO_4) were approximately half that of plants treated with 60 mM Na_2SO_4 (Fig. 6.3.4), with the exception of stem tissue, which had nearly equal SO_4 concentrations in plants from these treatments. Similar to Cl and Na, root SO_4 did not significantly increase after 3 weeks of treatment.

Root K concentration was similarly reduced by all salt treatments (Fig. 6.3.5). Potassium concentrations of stems, after 5 weeks of treatment, were significantly reduced by all salt treatments, but were reduced to a greater extent by treatments containing Na_2SO_4. In old needles, K concentrations were slightly reduced by treatment with Na_2SO_4, and significantly increased in 120 mM NaCl-treated plants. For new needles,

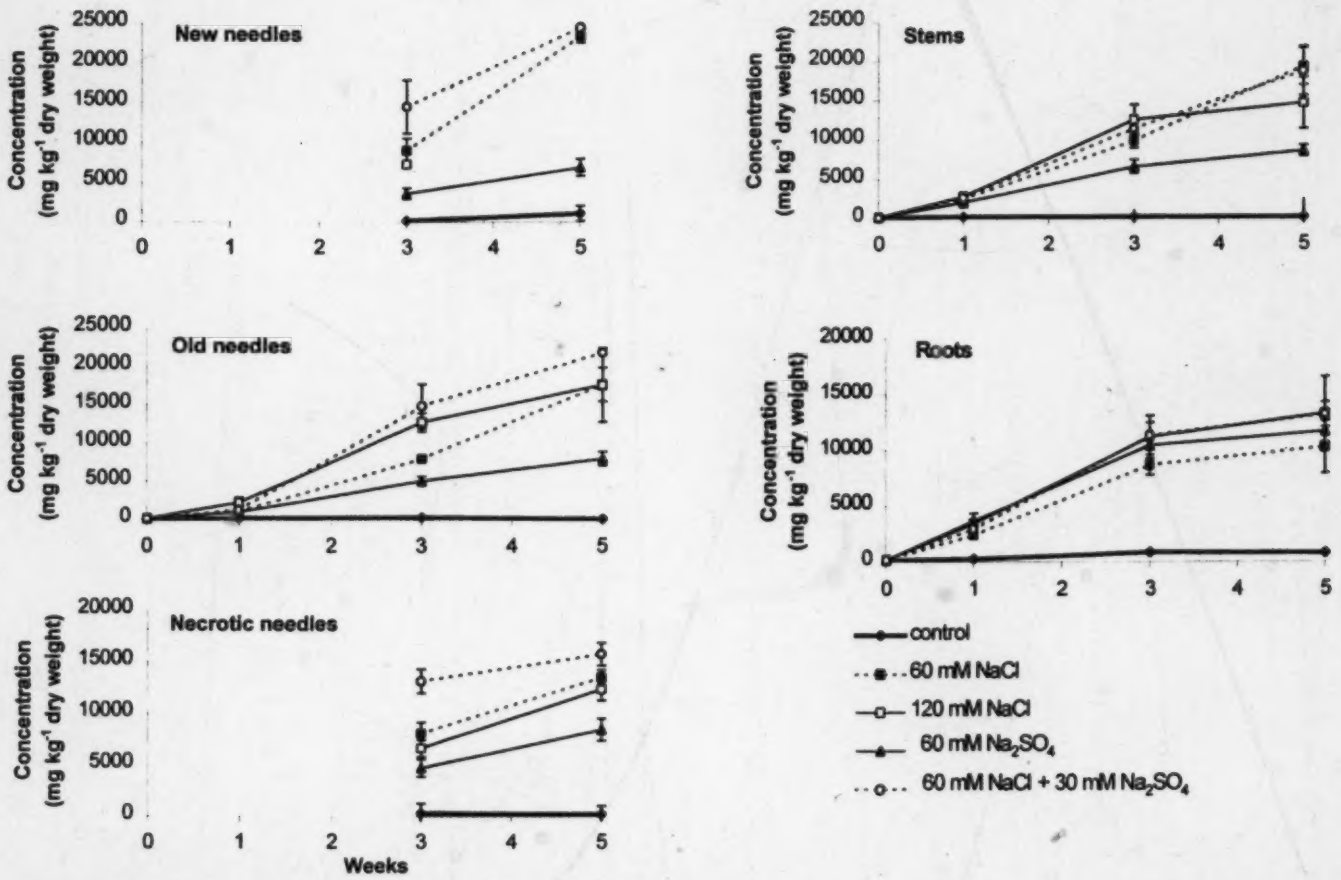

Fig. 6.3.2. Sodium concentration in new needles, old needles, necrotic needles, stem, and roots of jack pine treated for 5 weeks with salt solution. Bars represent ± SE.

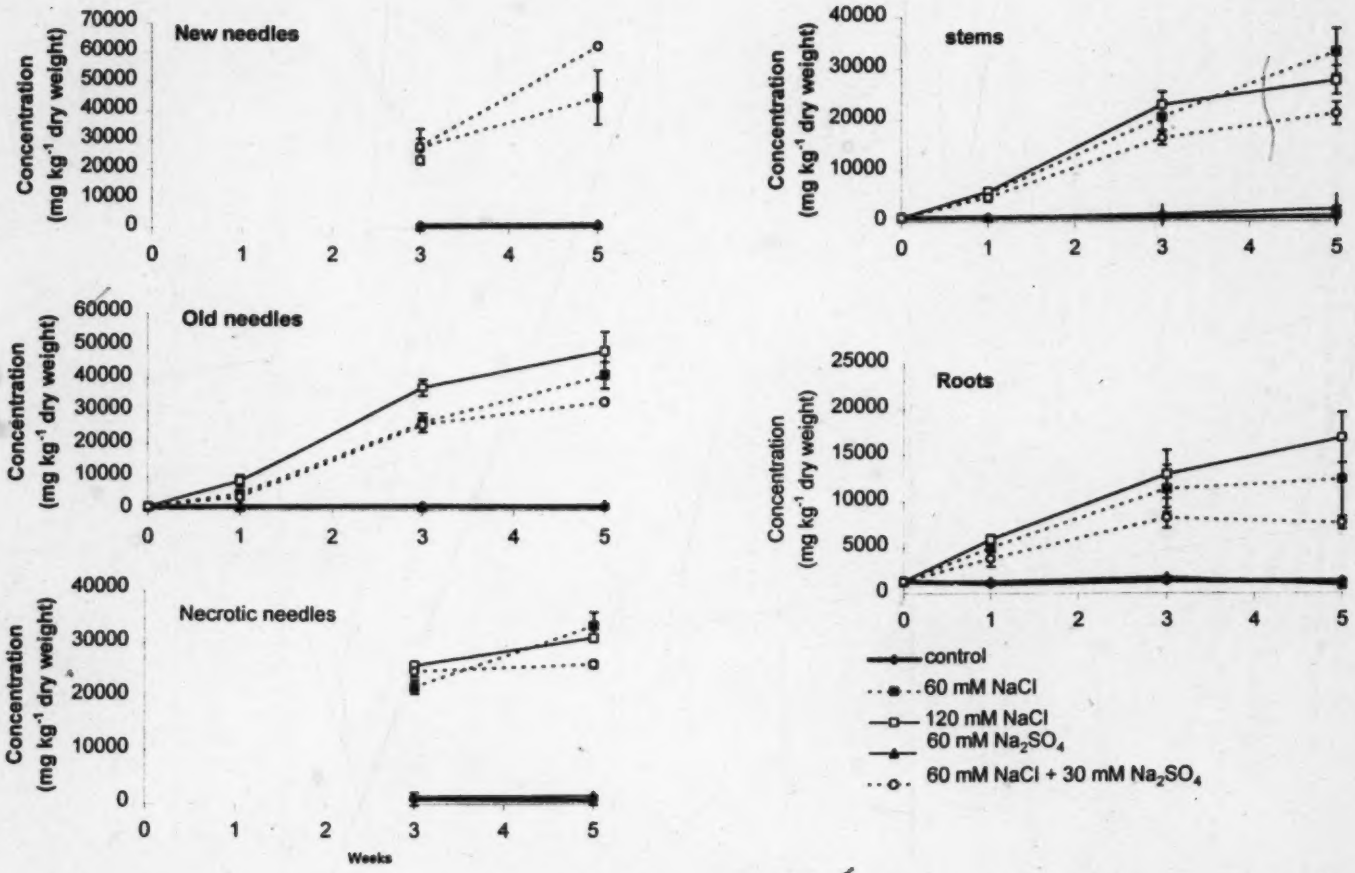

Fig. 6.3.3. Chloride concentration in new needles, old needles, necrotic needles, stem, and roots of jack pine treated for 5 weeks with salt solution. Bars represent ± SE.

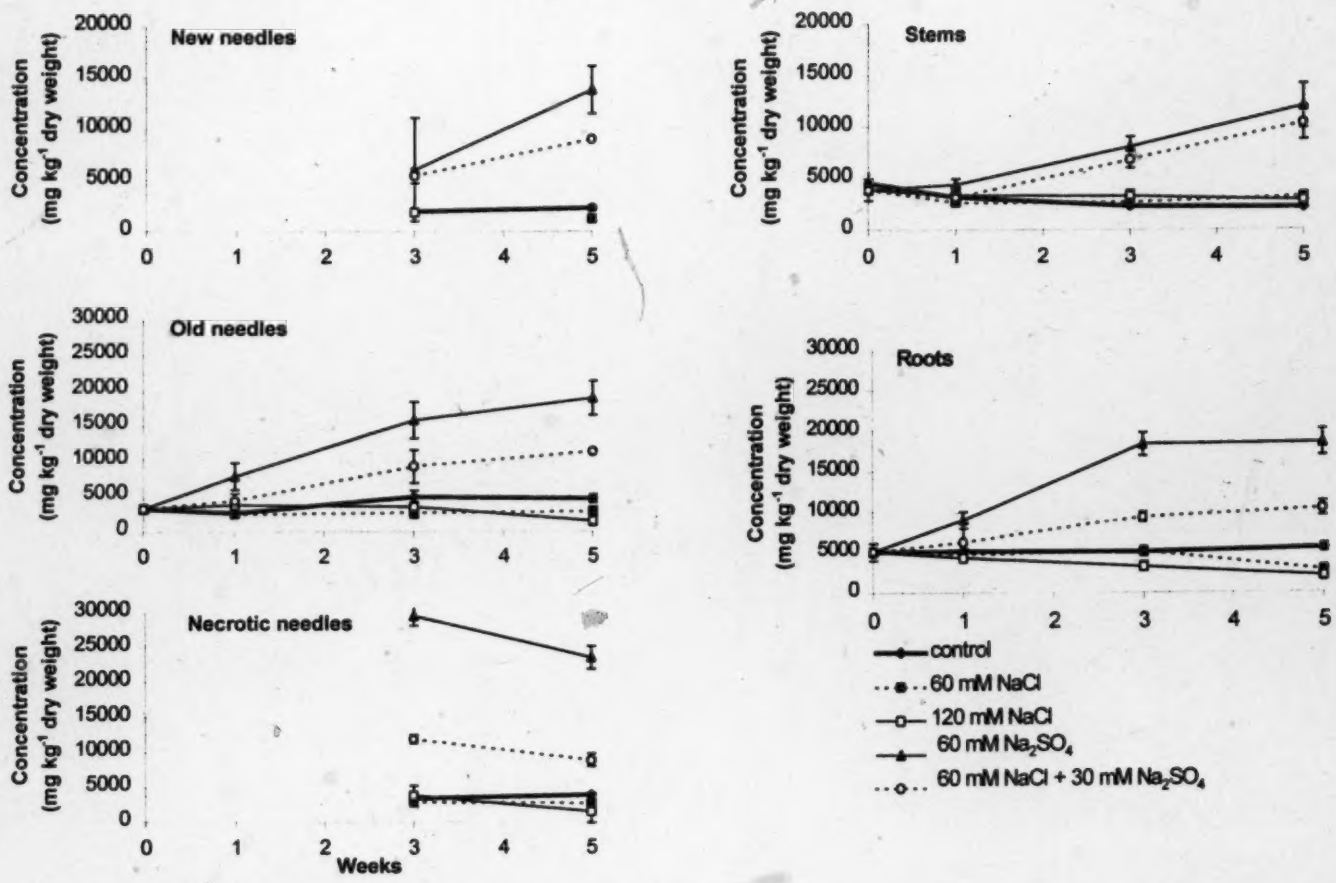

Fig. 6.3.4. Sulfate concentration in new needles, old needles, necrotic needles, stem, and roots of jack pine treated for 5 weeks with salt solution. Bars represent ± SE.

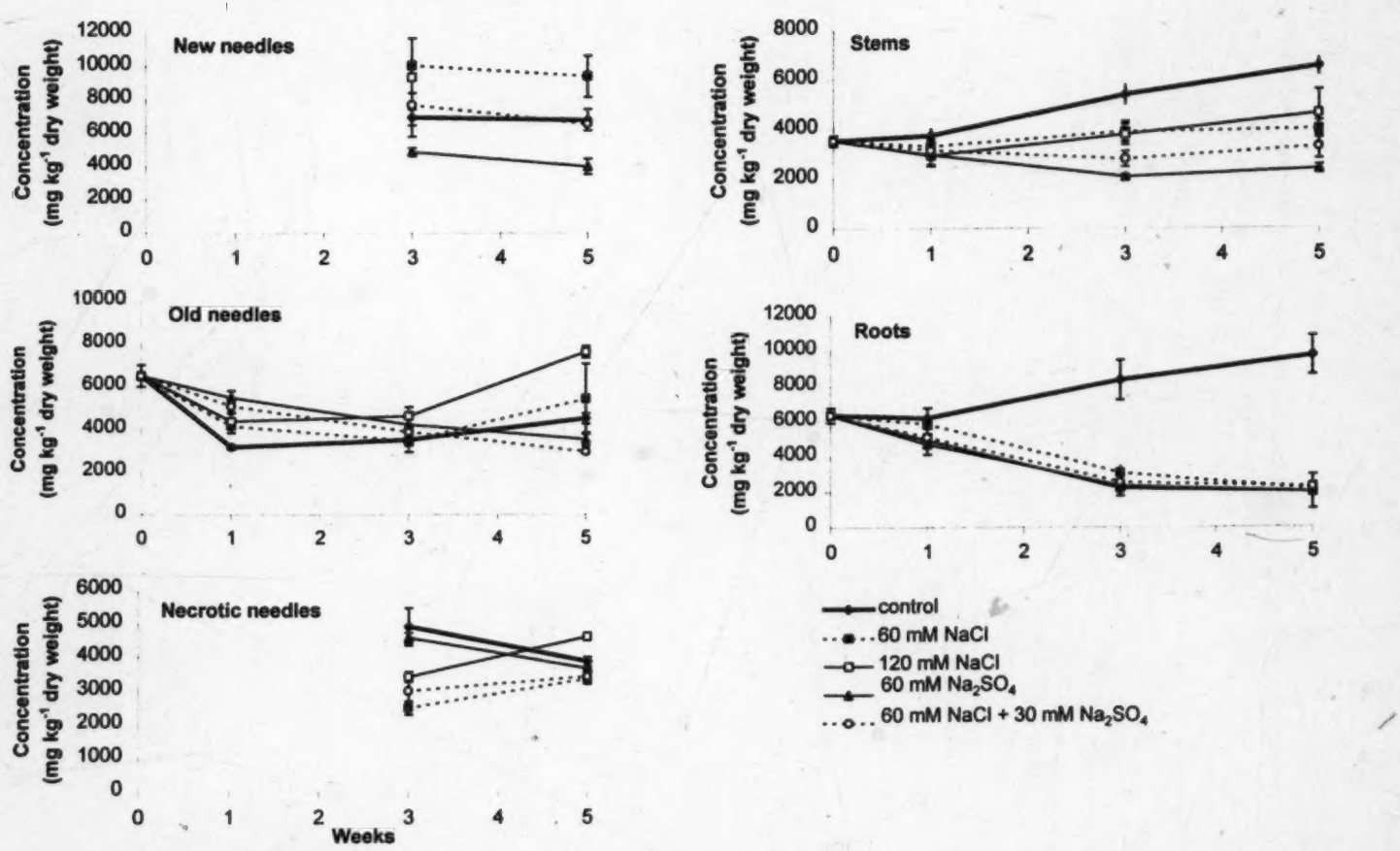

Fig. 6.3.5. Potassium concentration in new needles, old needles, necrotic needles, stem, and roots of jack pine treated for 5 weeks with salt solution. Bars represent ± SE.

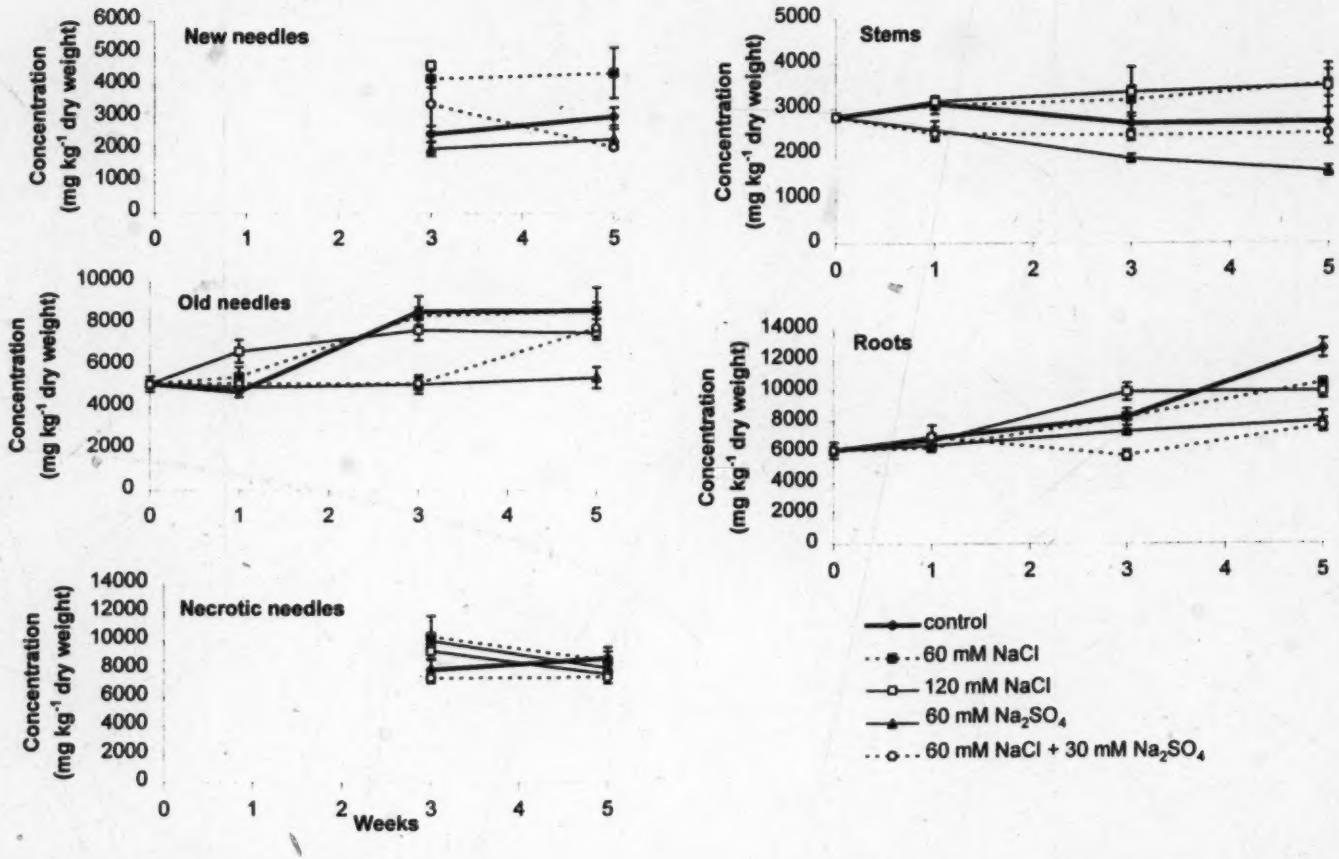

Fig. 6.3.6. Calcium concentration in new needles, old needles, necrotic needles, stem and roots of jack pine treated for 5 weeks with salt solution. Bars represent ± SE.

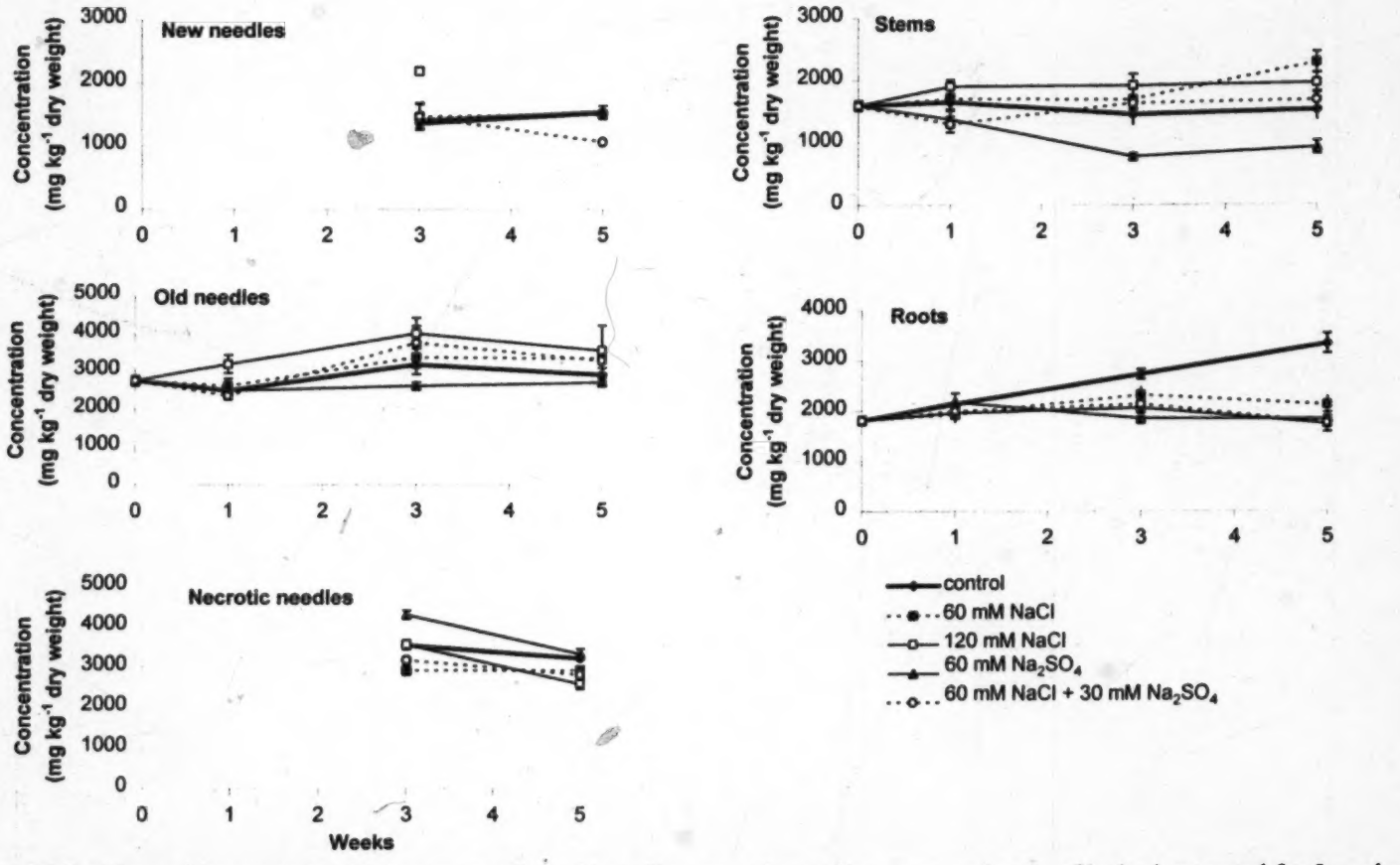

Fig. 6.3.7. Magnesium concentration in new needles, old needles, necrotic needles, stem, and roots of jack pine treated for 5 weeks with salt solution. Bars represent ± SE.

the reduced K concentration in plants treated with 60 mM Na_2SO_4 was significant, while other treatments did not differ significantly from controls.

Calcium concentrations in roots of control plants increased over the 5-week treatment period (Fig. 6.3.6). Roots of treated plants also showed an increase in Ca concentration at the end of 5 weeks, but this increase was less than control plants, with SO_4 treatments showing significantly lower concentrations than plants treated with 60 mM or 120 mM NaCl. In new needles treated with 60 mM or 120 mM NaCl, Ca concentrations were significantly higher than plants receiving SO_4 or control treatments. Stem Ca was significantly reduced only after 5 weeks of treatment with 60 mM Na_2SO_4, and old needles from this treatment did not show an increase in tissue concentration over time, as was seen in other treatments (Fig. 6.3.6).

As seen for Ca, Mg concentrations did not increase over time in roots of salt-treated plants, and were significantly reduced in stems of plants treated with 60 mM Na_2SO_4 (Fig 6.3.7). However, root Mg concentrations did not differ significantly between treatments and no significant effect of salt treatment was detected in needles.

6.3.3 Ion uptake and root-to-shoot transport rates

Sodium uptake and root-to-shoot transport rates were relatively low during the first week in all treatments, but increased between the first and third week of treatment (Fig. 6.3.8). Between 3 and 5 weeks, the rate of Na transport continued to increase while uptake decreased slightly, and a large degree of variation was seen between individuals. At week 3, both uptake and transport rates of plants treated with mixed salt were significantly higher than those of plants receiving the same Na level in the 60 mM Na_2SO_4 treatment. As seen for Na, Cl uptake and transport increased between the first and third week of treatment (Fig. 6.3.9). No difference in Cl uptake or transport rate was found between plants treated with 60 mM or 120 mM Cl. Sulfate uptake rate was much lower than that of Cl. Sulfate uptake decreased over time in plants treated with 60 mM Na_2SO_4, while in the mixed salt treatment it showed the same pattern as Na and Cl, with a large increase seen between 1 and 3 weeks (Fig. 6.3.10). Root to shoot transport did not differ significantly between treatments containing SO_4. In the first week of treatment

K uptake rate and root-to-shoot transport rate were negative in all treatments including the control (Fig. 6.3.11). Uptake and transport were positive in the control plants over the 1-3 week and 3-5 week intervals, while these remained negative or showed small positive values in all salt-treated plants.

Uptake and transport rates for Ca and Mg did not change significantly over time, therefore, the rates for all time intervals were combined for analysis. Calcium uptake was lower in all salt-treated plants than in control plants, with this reduction being statistically significant in plants treated with 60 mM Na_2SO_4 or mixed salt (Fig. 6.3.12a). Some root-to-shoot transport of Ca was maintained in these treatments, however, and differences between treatments were not significant. Magnesium uptake was also reduced by salt treatments, and was most affected by 60 mM Na_2SO_4 treatment where mean values were around zero (Fig. 6.3.12b). While less than half of absorbed Mg was transported to the shoots in control plants, root-to-shoot transport rates nearly equaled uptake rates in salt-treated plants.

6.3.4 Electrolyte leakage

Electrolyte leakage of stems and needles of control plants, over a 5-hour incubation period, was approximately 8% of total electrolytes. For most treatments, a small increase in needle and stem electrolyte leakage was measured after 1 week (Fig. 6.3.13). Electrolyte leakage in needles of plants treated with 60 mM Na_2SO_4 increased in a linear manner over time, while stems did not show a significant increase in electrolyte leakage after 5 weeks of treatment. Plants treated with 60 mM NaCl and 30 mM Na_2SO_4 + 60 mM NaCl exhibited the same trends, with needle leakage increasing dramatically between 1 and 3 weeks, and stem leakage increasing between 3 and 5 weeks. Plants treated with 120 mM NaCl showed a large increase in needle leakage after only 1 week, with visible injury appearing 3 days later. Sufficient growth of new needles in control, 60 mM NaCl and 60 mM Na_2SO_4 treatments allowed determination of electrolyte leakage in plants harvested after 5 weeks of treatment, and this was found to be 344% of control plants for plants treated with NaCl, and 148% for those treated with Na_2SO_4.

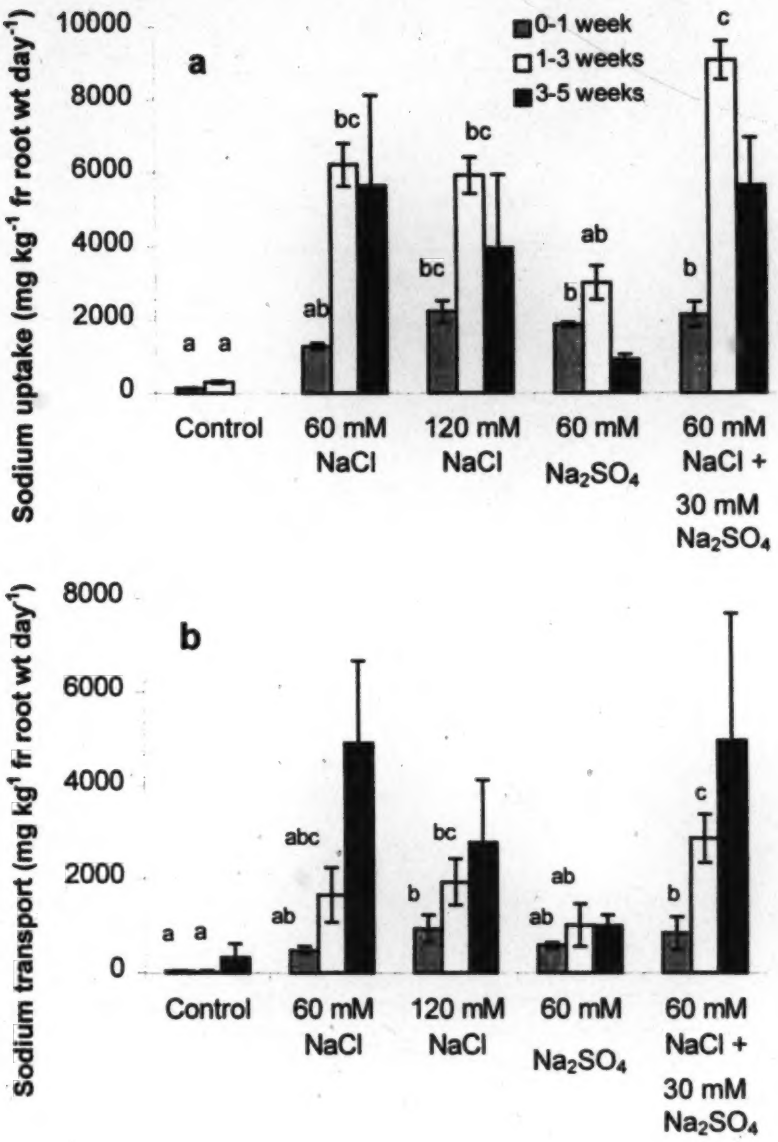

Fig. 6.3.8. a) Sodium uptake rate (Jt) and b) Na root-to-shoot transport rate (Js) based on average fresh root weight, over three time intervals in plants treated with salt. Bars represent $\pm$ SE. Different letters represent significant differences between treatments within a time interval at $\alpha = 0.05$.

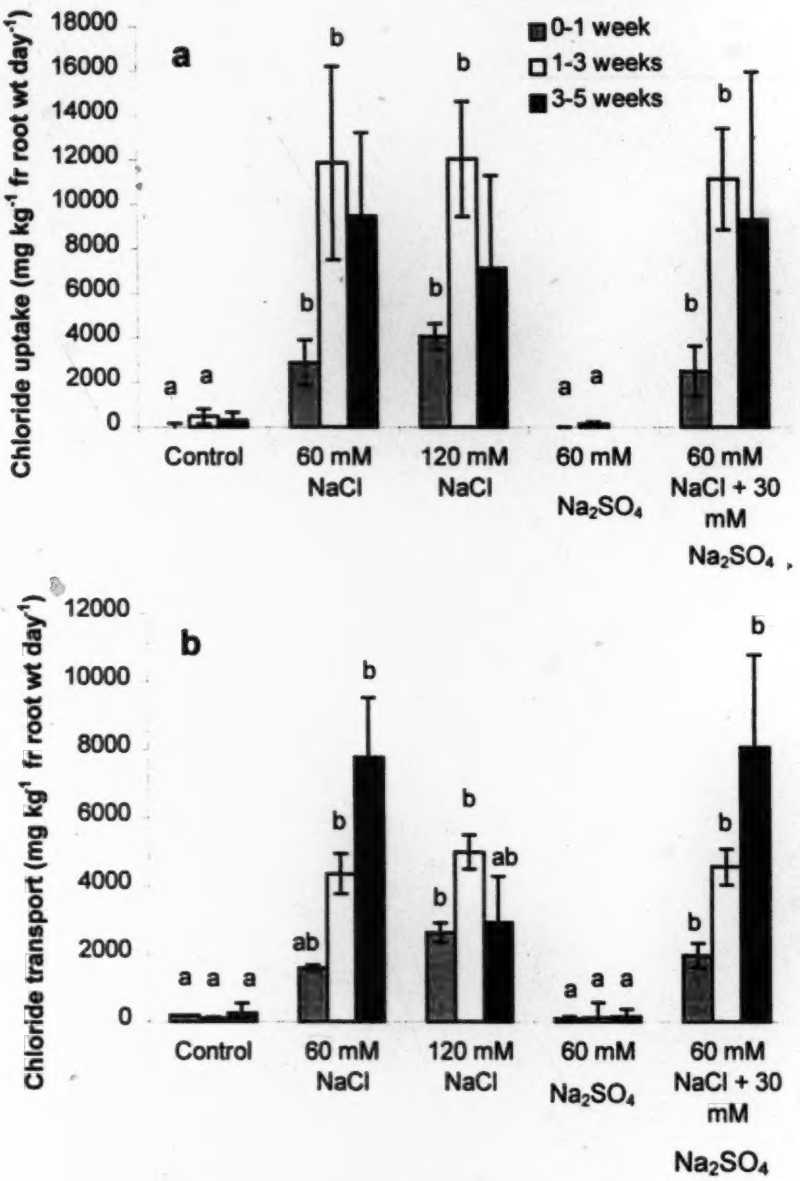

Fig. 6.3.9. a) Cl uptake rate (Jt) and b) Cl root-to-shoot transport rate (Js) based on average fresh root weight, over three time intervals in plants treated with salt. Bars represent ± SE. Different letters represent significant differences between treatments within a time interval at $\alpha = 0.05$.

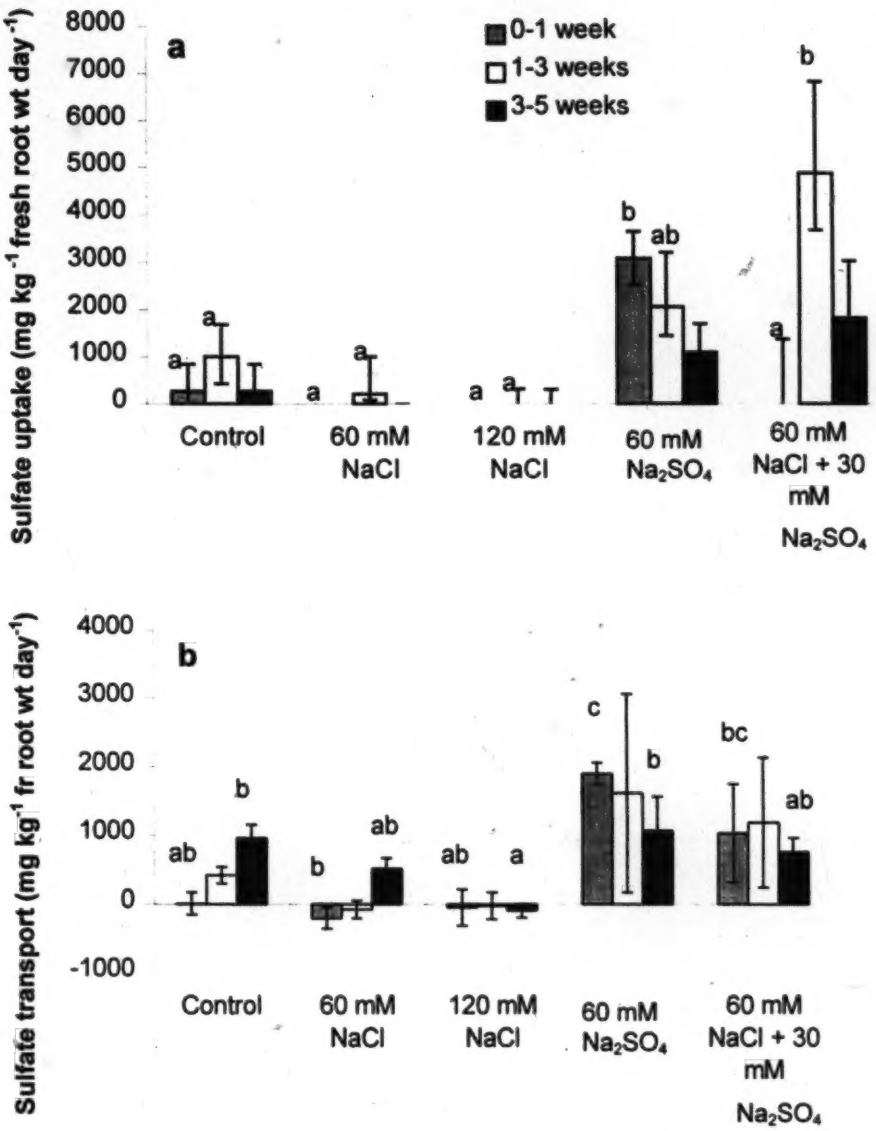

Fig. 6.3.10. a) Sulfate uptake rate (Jt) and b) SO_4 root-to-shoot transport rate (Js) based on average fresh root weight, over three time intervals in plants treated with salt. Bars represent $\pm$ SE. Different letters represent significant differences between treatments within a time interval at $\alpha = 0.05$.

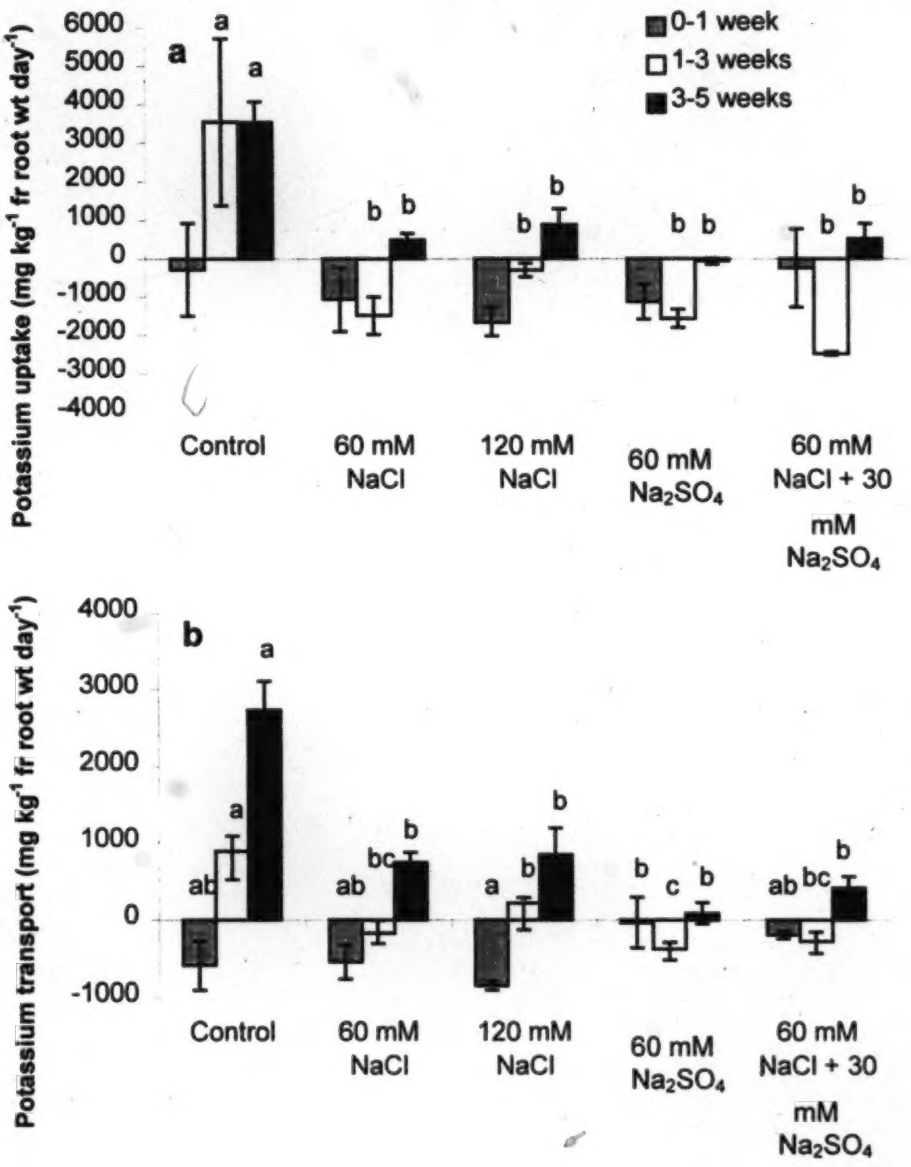

Fig. 6.3.11. a) Potassium uptake rate (Jt) and b) K root-to-shoot transport rate (Js) based on average fresh root weight, over three time intervals in plants treated with salt solution. Bars represent ± SE. Different letters represent significant differences between treatments within a time interval at $\alpha = 0.05$.

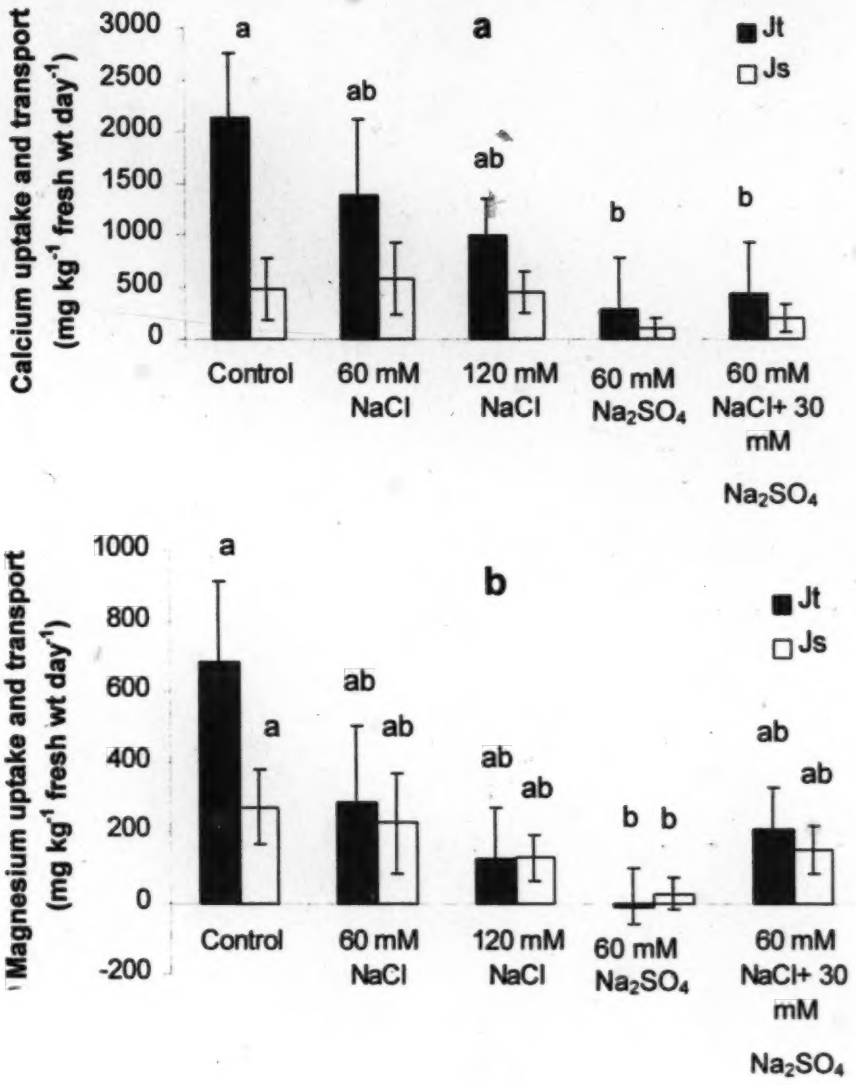

Fig. 6.3.12. a) Calcium uptake rate (Jt) and root-to-shoot transport rate (Js), and b) Mg uptake rate (Jt) and root-to-shoot transport rate (Js) based on average fresh root weight, and averaged over three time intervals in plants treated with salt. Bars represent $\pm$ SE. Different letters represent significant differences between treatments at $\alpha = 0.05$.

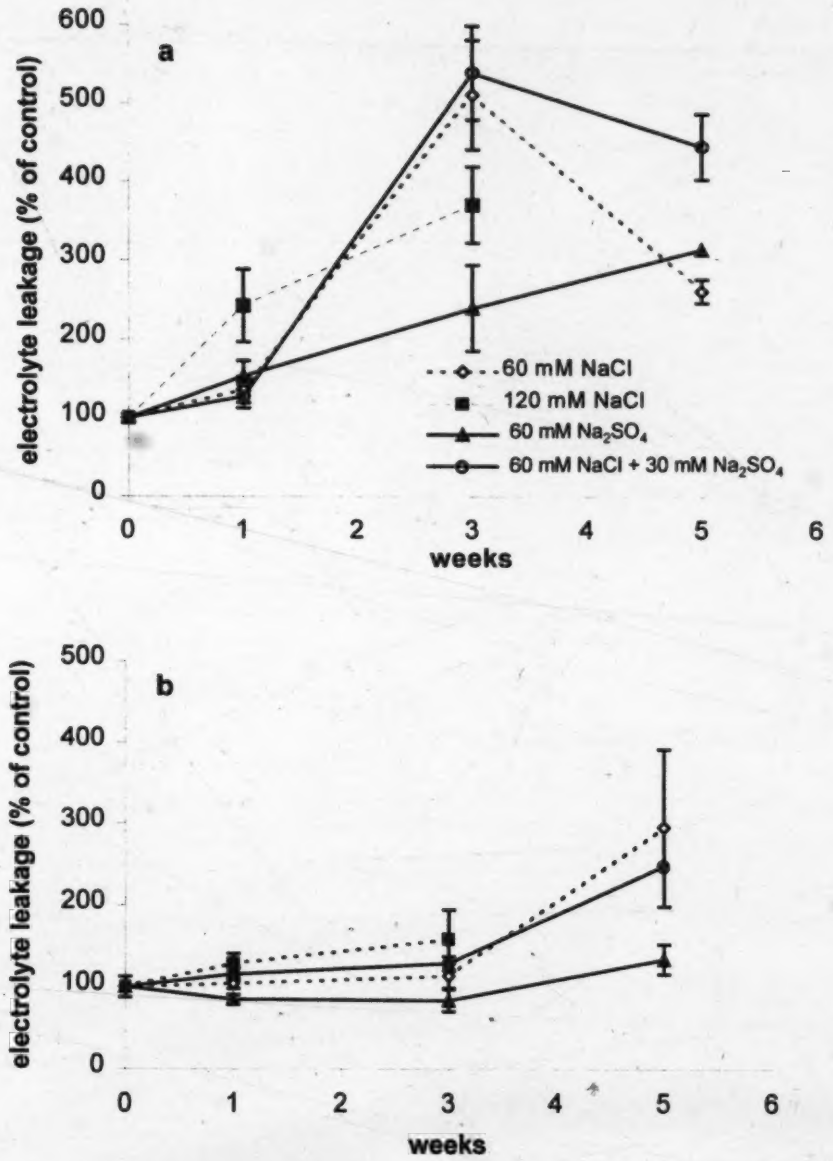

Fig. 6.3.13. Relative electrolyte leakage of needles (a) and stems (b) of jack pine seedlings treated with salt for 5 weeks. Bars indicate ± SE.

Table 6.3.1. Pearson correlation coefficients (R) of electrolyte leakage (EL) of stems and needles, needle necrosis (% of needle dry weight), and tissue Na and Cl concentrations of seedlings treated with solutions containing 60 mM Cl (60 mM NaCl and 60 mM NaCl + 30 mM Na_2SO_4), SO_4 alone (60 mM Na_2SO_4), or all treatments including control seedlings receiving no salts.

	Tissue conc.	EL stem	EL needles	% necrosis
Chloride treatments	Cl	.687**	.821**	.819**
N = 25	Na	.841 **	.795**	.355
	% necrosis	.623	.810**	
Sulfate treatment	Cl	-.018	-.136	.015
N = 17	Na	-.127	.206	.559
	% necrosis	.467	.605	
All treatments	Cl	.695**	.674**	.917**
N = 56	Na	.641**	.563**	.767**
	% necrosis	.710**	.895**	

** indicates significance at the 0.001 level

When analyzed across all treatments, and across 60 mM Cl treatments, electrolyte leakage of stems and needles showed significant positive correlation with tissue Cl and Na concentrations (Table 6.3.1). However in plants treated with 60 mM Na_2SO_4, neither stem nor needle leakage was related to tissue Na. Across all treatments, both Na and Cl were correlated with needle necrosis, while among Cl treatments only Na related to necrosis. In plants treated with 60 mM Na_2SO_4, Na was not related to necrosis. Needle necrosis was significantly related to needle electrolyte leakage across all treatments, and among Cl treatments, but this relationship was not significant in Na_2SO_4-treated plants.

6.4 Discussion

Growth of all tissues was similarly depressed by all treatments, and neither salt type nor salt concentration appear to be primary factors in growth inhibition. Reductions in growth and yield are commonly observed under salt conditions. However, evidence suggests that growth inhibition is not a result of exposure to low osmotic potentials (Munns and Termatt, 1986). The effects of salt type on growth vary with species. In sorghum (*Sorghum bicolor* (L.) Moench.), growth was more reduced by Na_2SO_4 than by isomolar NaCl, and roots were affected than shoots (Khan et al., 1995), while salt level had a much greater effect than salt type in chickpea (Lauter and Munns, 1986). The significant growth reduction observed in this study suggests that jack pine is relatively sensitive to salts. In maritime pine (*Pinus pinaster* Ait.), growth was reduced only by NaCl treatments of 150 mM or higher (Saur et al., 1995).

Delayed transport of Na and Cl to shoots indicates some retention of these ions in roots. Radiata pine (*Pinus radiata*) also appears able to limit uptake of translocation of Na (Myers et al., 1998). After 3 weeks of treatment, similar levels of Na were found in roots of all salt treatments, and little further accumulation of Na was seen, suggesting a possible saturation of the root tissue at approximately 10000-12000 mg Na kg^{-1} DW root. Transport rates approximately equaled uptake rates over the 3-5 week interval. Between 1 and 3 weeks, concentrations of both Cl and Na in stem and needle tissue rose, reflecting the increase in root-to-shoot translocation. A similar increase in transport rate with

duration of NaCl treatment was found in a salt sensitive genotype of olive (*Olea europaea* L.) (Tattini, 1994). The increase in Cl and Na transport over time may be due to reduced ability of root to sequester the ions. Slightly higher Na uptake transport rates under Cl salinity lead to a significantly greater accumulation of Na in shoot tissues than was seen in plants treated with 60 mM Na_2SO_4 (Fig. 6.3.8, Fig. 6.3.2). Similar results have been found in dogwood (Renault et al. ,2001) and in sorghum (*Sorghum bicolor* (L.) Moench.) (Khan et al., 1995).

Chloride uptake and transport followed the same pattern as Na, and differed little between Cl treatments (Fig. 6.3.9). These parameters were apparently independent of external concentration, suggesting that rates are limited by the number of channels or carriers. This agrees with the generally accepted model of Cl transport across the plasma membrane as an active process (Maas and Ogata, 1972; Tyerman and Skerrett, 1999). The retention of Cl in root tissue has been reported for NaCl-treated oak (*Quercus rober* L.) (Alaoui-Sosse et al., 1998), and leaf Cl also appears to be controlled by restriction of root to shoot transport in soybean (*Glycine max* L.) (Grattan and Maas, 1985) and in some citrus rootstocks (Grieve and Walker, 1983). However, many species do not appear to be able to effectively regulate Cl entry into the shoot and accumulate Cl in greater amounts than Na (Jacoby, 1994). This was also observed in the present study in jack pine, after the first week of treatment (Fig. 6.3.2, 6.3.3). Since concentrations of these ions in necrotic needle tissue were similar, or slightly lower to that in living needles, neither Na nor Cl appears to have been preferentially transported to dead or dying tissues.

In contrast with Cl transport, SO_4 concentrations in all roots and needles increased with treatment concentration (Fig. 6.3.4). Concentrations in plants treated with mixed salt (30 mM SO_4) were half of those in plants treated with 60 mM SO_4, likely the result of lower initial uptake and transport rates. My results did not show any delay of transport to the shoot, as was seen for Na and Cl, and transport and tissue concentrations were generally about half of the values recorded for Cl, with the exception of necrotic tissue. Sulfate is thought to be less readily absorbed than Cl (Bernstein, 1975). In highbush blueberry *(Vaccinium corymbosum)*, SO_4 was also found to accumulate in tissues to a much lesser extent than Cl (Muralitharan et al., 1992).

Electrolyte leakage of needles increased in a slow, linear pattern over time in plants treated with 60 mM Na_2SO_4, but increased rapidly over the 1-3 week time interval in plants treated with 60 mM NaCl (Fig. 6.3.13). Reduced electrolyte leakage values for 60 mM NaCl and mixed salt treatments at 5 weeks, and only a slight increase for 120 mM NaCl at 3 weeks may have been due to more resistant plants being measured at this time. Many plants in the high-salt treatments were severely injured, and those showing more than 75% necrosis were not used for electrolyte leakage measurements. A change in K uptake and transport from negative to positive values, and reduced Na uptake was found at 5 weeks, and suggests that reduced electrolyte leakage could also be due to adaptive responses or recovery. The apparent increase in membrane leakage seems to parallel the increase in salt content of the shoot in both NaCl and Na_2SO_4 treatments, and the movement of Na is closely associated with movement of the anion. The observed injury could be explained by possible initial damage to the plasma membrane by Na, since high Na levels can result in membrane depolarization and K efflux (Jacoby, 1994). It is also plausible that Cl may be responsible for initial damage to the plasma membrane. Chloride has been found to alter membrane lipid composition resulting in increased membrane permeability (Kuiper 1968), and this leakage has been proposed as a mechanism of Cl-induced injury (Bernstein, 1975). In either case, increasing permeability results in a reduced ability of cells to compartmentalize potentially toxic ions, and secondary damage to the cell may then result from elevated cytoplasmic levels of the counter-ion. This was suggested by Martin and Koebner (1995), who found that the effects of Na and Cl together are greater than the effects attributable to either ion alone. Membrane leakage of root cells may result in increased uptake of salt ions, and their subsequent translocation to the shoot.

The possibility of Na-induced injury is supported by the similarity between electrolyte leakage and Na accumulation patterns, as it is reasonable that Na moved more quickly with the highly mobile Cl^- ion, or more slowly with the SO_4^{2-} ion, as a charge balance. When analyzed across all treatments, both Na and Cl were correlated with electrolyte leakage and with needle necrosis (Table 6.3.1). However when the Na_2SO_4-treated plants were analyzed alone, no relationship was found between tissue Na concentration and electrolyte leakage. Furthermore, while accumulation of Na in all shoot

tissues was higher in the presence of Cl, Cl transport did not appear to be dependent on treatment Na concentration (Fig. 6.3.9). In fact, given the same treatment concentration of Cl, accumulation of Cl in all tissues was lower in the mixed salt treatment (60 mM NaCl + 30 mM Na_2SO_4), which contained twice the amount of Na than the 60 mM NaCl treatment (Fig. Fig. 6.3.3). Previous studies have shown the mechanisms of Na and Cl transport to be independent and not directly coupled (Maas and Ogata, 1972; Grieve and Walker, 1983). The accumulation of concentrations of Cl higher than 5000 mg kg^{-1} in the shoot preceded the accumulation of Na to similar levels. My results therefore suggest that initial injury, measured as an increase in electrolyte leakage, is more likely due to Cl than to Na in jack pine treated with NaCl. Sodium entry into a cell is thought to be an active process, with net Na influx being regulated by active efflux through Na^+/H^+ antiporters (Blumwald et al., 2000). A disadvantage of my method for measuring electrolyte leakage is the possibility of mistakenly interpreting active Na efflux as leakage. However if this were the case, I would expect to see a strong relationship between tissue Na concentration and electrolyte leakage. Such a relationship was not present in plants treated with 60 mM Na_2SO_4.

Reductions in K uptake and transport were seen in all salt treatments, but were more pronounced in the 60 mM Na_2SO_4 treatment. Net uptake over the 5-week treatment period was negative in all salt treatments indicating K efflux, and tissue concentrations decreased in the root and stem. Efflux of K may act to maintain the electrical potential across the plasma membrane and therefore be an adaptive response to high external ion levels (Cumming and Taylor, 1990). Needle K concentrations of plants treated with 60 mM or 120 mM NaCl were higher than that of control plants after 5 weeks. Decreased root K and increased leaf K concentrations have also been observed in bean (*Phaseolus vulgaris*) (Carbonell-Barraching et al., 1997) and radiata pine (Sands and Clark, 1976) treated with NaCl. Increased translocation of K to young tissues under NaCl salinity has been demonstrated in castor bean (*Ricinus communis* L.) (Jeschke and Wolf, 1988), and higher levels of K cycling were found in the more tolerant castor bean (*Ricinus communis* L.) and barley (*Hordeum vulgare* L.) than in the salt sensitive white lupin (*Lupinus albus* L.) (Jeschke and Pate, 1991).

The uptake and transport of Ca and Mg were affected more by 60 mM Na_2SO_4 than by 120 mM NaCl treatment containing the same Na level, suggesting effects are not limited to simple competitive uptake with Na. The effects of Ca deficiency are often seen in crop plants under saline conditions (Grattan and Grieve, 1999). Calcium translocation in barley was inhibited by Na_2SO_4, NaCl, and mannitol, and the authors attributed reduced translocation to both osmotic potential of the treatment solution, and a reduction in symplastic flow of Ca across the root (Halperin et al., 1997). However, plant response varies widely between species and genotype (Grattan and Grieve, 1994), and the effects of SO_4 salinity have not been widely studied. Although I found Mg uptake of treated plants to be less than half that of control plants, translocations rates were less affected, resulting in reduced root Mg concentrations while needle Mg was little affected. Similarly, little change in needle Mg concentration was found in needles of radiata pine treated with NaCl (Sands and Clark, 1976), or with a mixture of Cl salts (Myers et al., 1998).

In conclusion, jack pine appears to have some ability to retain Na and Cl in the roots, but this ability is overcome with prolonged exposure to high NaCl levels. Sodium uptake and translocation was closely tied to that of the accompanying anion, and electrolyte leakage increased in parallel with needle salt content. The increase in leakage was rapid in plants treated with NaCl, and I suggest that it is the direct result of the Cl ion. Na uptake was greater from NaCl compared with Na_2SO_4 treatment, while the reverse was found for Ca and Mg.

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Chapter VII

Sodium uptake is not dependant on transpiration in jack pine (*Pinus banksiana*)

7.1 Introduction

Land area affected by salinity is increasing throughout the world, creating a great economic impact, particularly on the agricultural industry. In temperate regions, woody plants are most often exposed to salinity from anthropogenic sources such as road salts or mining activity. Two direct effects of salinity in the plant environment are osmotic stress and ion toxicity, although the relative importance of these stresses may vary with plant species, duration of treatment (Munns and Termaat, 1986), and salt composition (Bernstein, 1975). Ion toxicity results from high tissue concentrations of salt ions, and restriction of Na or Cl translocation to the shoot is one means by which plants may tolerate salinity, as reported for salt tolerant cultivars of olive (*Olea europaea* L.) (Tattini, 1994) and mango (*Mangifera indica* L.) (Schmutz and Ludders, 1998). Plant injury is, in some cases, directly related to shoot ion concentration. Needle Cl and Na were related to necrosis of red pine (*Pinus resinosa* Aiton) (Sucoff et al., 1975) and Eastern white pine (*Pinus strobus* L.) (Hall and Hofstra, 1972).

My studies of jack pine (*Pinus banksiana* Lamb.) have shown shoot Na concentrations to be related to needle necrosis in seedlings treated with NaCl, but not in those treated with Na_2SO_4, and suggest that Cl toxicity is greater than that of Na in this species (Chapter VI). Seedling growth under saline conditions was not related to shoot elemental composition, suggesting that growth reduction was not a direct result of ion toxicity or nutrient deficiency. Growth reduction could potentially be affected by changes in plant water relations and reduced transpiration rates, suggested by the greater rate of root-to-shoot Na transport in the presence of Cl. Higher accumulations of Na in shoot tissue under NaCl treatment than Na_2SO_4 treatment have also been reported for dogwood (*Cornus stolonifera* Michx.) (Renault et al. 2001), and highbush blueberry (*Vaccinium corymbosum* L.) (Muralitharan et al., 1992). Although these high tissue

concentrations of ions may have negative effects, they may also provide osmotic adjustment, as inorganic ions are the main contributor to reduced osmotic potential (Greenway and Munns, 1980; Rodriguez et al., 1997).

Although the effects of NaCl on plants are well known, relativley few studies have compared the effects of NaCl and Na_2SO_4. Shoot tissue ion content is dependant on both root-to-shoot transport rate and shoot growth rate, as plant growth provides new tissue with the potential for ion sequestration (Volkmar et al., 1997). Both ion transport and growth are potentially affected by transpiration rate. In theory, there is a positive relationship between transpiration and solute transport (Dalton et al., 1975; Dalton et al., 2000). In previous studies of jack pine (Chapter VI) and dogwood (Renault et al., 2001) where a lower accumulation of Na was observed in Na_2SO_4-treated plants than in NaCl-treated plants, measurements of transpiration were not make. The focus of the current study, therefore, is to determine whether this difference in Na uptake is due to corresponding differences in transpiration rates between jack pine treated with these two salts. I have used jack pine seedlings in solution culture to investigate the water and ion uptake of jack pine seedlings under both NaCl and Na_2SO_4 salinity, and to examine the relationships between transpiration, water potential, root hydraulic conductivity and the uptake of major cations and anions. I have measured the total volume of water transpired by individual plants over a three-week period to test the hypothesis that root-to-shoot ion transport rates are directly related to transpiration rates.

7.2 Materials and Methods

7.2.1 Plant material

Jack pine (*Pinus banksiana* Lamb.) cones were collected approximately 60 km north of Fort McMurray, Alberta (57°05.95N 111°38.90W). Seeds were extracted, germinated in Petri dishes, and planted when emerged radicles were approximately 10-mm long. Planting media was a mixture of quartz/feldspar sand and peat, in a ratio of 1:1 by volume, in the cavities of "Spencer-Lemaire rootrainers" (Stuewe and Sons, Inc.,

Corvallis) providing a rooting volume of 90 ml per seedling. Seedlings were grown in a controlled environment chamber at 45% relative humidity, 24°/18° C (day/night) temperature and 18-hour photoperiod with 325 ± 25 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by cool white fluorescent (Sylvania) lamps. Plants were watered three times a week with a nutrient solution (80 mg L^{-1} N, 60 mg L^{-1} P, 104 mg L^{-1} K, 100 mg L^{-1} Ca, 60 mg L^{-1} Mg, 79 mg L^{-1} S, 3 mg L^{-1} Fe, 0.40 mg L^{-1} Mn, 0.25 mg L^{-1} B, 0.14 mg L^{-1} Zn, 0.50 mg L^{-1} Cu, 0.10 mg L^{-1} Mo, pH 5.0 to 5.3) recommended for pine seedling production (Wood 1995).

7.2.2 Water relations

When seven-months-old, plants were removed from Spencer-Lemaire containers, and roots were gently washed with deionized water to remove sand and peat. Seedlings were placed into aerated solution culture (Fig. 7.2.2) consisting of the nutrient solution described above, and allowed seven days to recover before the start of treatments. Aeration was provided by an "Optima" aquarium pump (model 807, Rolf C. Hagen Inc., Montreal) which delivered air at a volume of 5000 cm^3 min^{-1}. Eight seedlings were weighed then transferred to each tub of aerated nutrient solution (control), or nutrient solution containing 60 mM NaCl, 120 mM NaCl, 60 mM Na_2SO_4, or 60 mM NaCl + 30 mM Na_2SO_4. The osmotic potentials of the treatment solutions were estimated based on the EC of the nutrient solution (Jurinak, 1990) and osmotic potential of the added salts at 25°C using the van't Hoff equation, using the assumption of complete solubility of the salts. At the start of the treatment period, three plants per replicate were harvested to provide baseline data. At the end of one week of treatment, three plants from each treatment and replicate were harvested, and the remainder of plants was harvested at the end of the 3-week treatment period.

Immediately prior to harvesting and one hour after the beginning of the daylight period, transpiration and diffusive resistance were measured on upper, fully expanded needles, using a steady-state porometer (Lambda Instruments Corp., Lincoln, NE, USA). Transpirational leaf area was estimated as projected leaf area using Sigma Scan 3.0 software (Jandel Scientific, San Rafael, CA). The shoot was excised 1 cm above the

uppermost lateral root, and shoot water potential was determined using a Scholander pressure chamber (PMS Instrument Co., Corvallis, OR, USA) (Scholander et al., 1965). Respiration of the excised root system was measured as oxygen uptake using a Clark-type electrode (Yellow Springs Instrument Co., Ohio) at a temperature of 25°C. The root system was placed in a dark, airtight vessel, in the same treatment solution from which it was harvested, and was continuously agitated with a magnetic stirrer. Oxygen content of the solution was recorded every 2 minutes for the first 10 minutes, and at 5-minute intervals for a further 10 minutes. Respiration was calculated as the slope of the regression line. Root hydraulic conductance (K_r) of each root system was measured by applying increasing pressure to the cut surface of the root using a high pressure flow meter (Tyree et al., 1995) (Dynamax Inc., Houston), and was determined from the slope of the regression line between 180 and 400 lb. pressure where the response was linear. Root hydraulic conductivity (Lpr) was calculated based on the fresh weight of the root system (Carvajal et al., 1996; Clarkson et al., 2000), as measurements of root surface area were thought to have a large degree of error. The experiment was replicated three times, in a completely randomized block design.

7.2.3 Ion uptake

A further twenty seedlings were transferred to solution culture as described above, and at the end of the one-week recovery period were weighed and transferred into 250 ml bottles with one plant per bottle, and 8 replicate bottles for each treatment solution. The plant stem was inserted into a one-holed rubber stopper, which was placed into the top of the bottle and sealed with modeling clay. The solution was aerated by an aquarium pump (4 bottles per pump operating at 5000 cm^3 min^{-1}) to an aeration stone at the bottom of the bottle, through tubing which sealed at the tubing/bottle junction (Fig. 7.2.3.1 and Fig. 7.3.2.2). A second tube provided air outflow. Air entering and leaving the bottle passed through a tube of calcium sulfate desiccant ("Drierite", W. A. Hammond Drierite Company Ltd., OH). Desiccant was changed daily, and evaporation from each bottle was determined from the beginning and end weights of the outflow desiccant. Daily transpiration was then calculated as the change in bottle weight less evaporation.

Fig. 7.2.2. Photograph of experimental set-up utilizing solution culture.

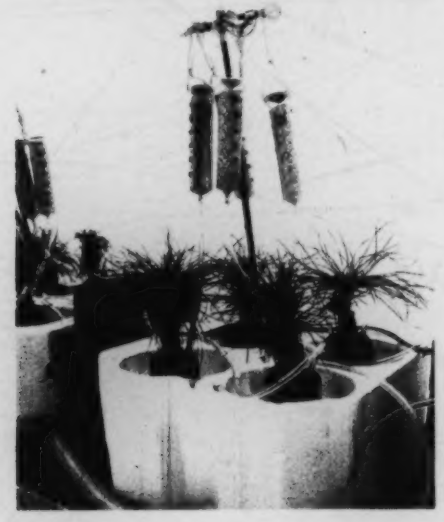

Fig. 7.2.3.1. Photograph of the sealed system experimental set-up.

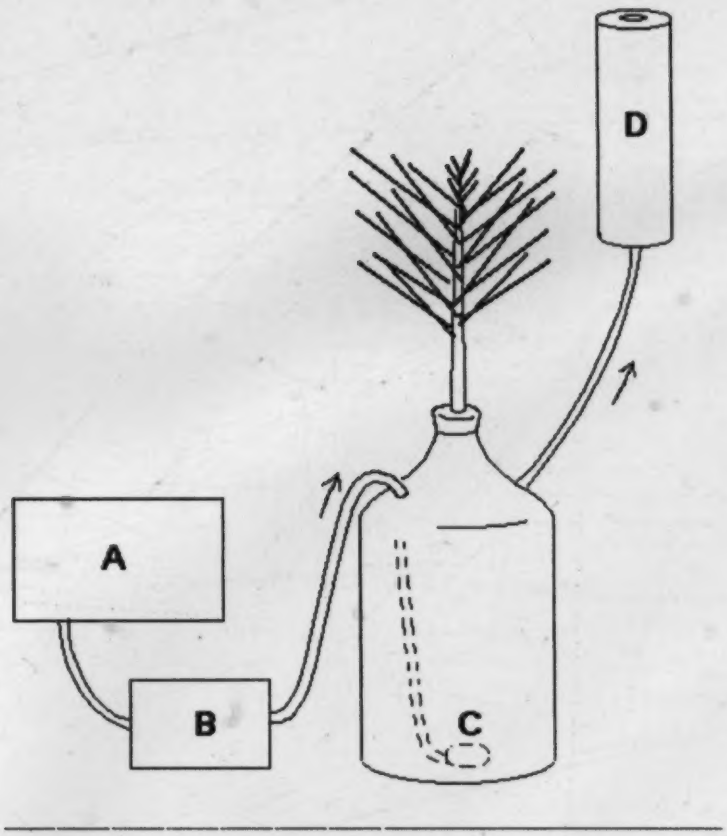

Fig. 7.2.3.2. Sealed system for the determination of total transpirational volume. A- Air pump, B- cylinder filled with dessicant, C- aquarium stone in bottle of treatment solution, D- cylinder of desiccant on air outflow line. Arrows indicate the direction of air flow.

At the end of the 3-week treatment period, plants were rinsed briefly in three changes of de-ionized water (3 x 30 sec.), separated into root and shoot, weighed, and ground to pass through a 2 mm sieve. Cation content was quantified using optical emission spectroscopy (ICP-OES) (Vista-PRO RL, Varian Analytical Instruments, Victoria, Australia) after microwave digestion of samples in nitric acid and filtration with 0.45 μm Millipore filters. Anion content was determined by ion chromatography (DI 300, Dionex Corporation, Sunnyvale, CA) on a filtered water sample obtained by combining two successive one-hour extractions of the sample with hot water. Root-to-shot ion transport was expressed as a function of total transpiration over the 3-week treatment period using the formula:

$$\text{flux} = ((m_{s2} - m_{s1})*W_{s2}) / (R_T * (T_2 - T_1))$$

Where m_{s1} represents the average shoot ion concentration at the beginning of the experiment (T_1), m_{s2} represents the ion concentration of a given shoot after 3 weeks of treatment (T_2), W_{s2} represents the dry weight of the shoot at the end of the treatment period, and R_T represents the total transpiration over the 3 weeks of treatment. The reflection coefficients (σ), where a value of 1 represents a membrane completely impermeable to a given solute and a value of 0 reflects complete permeability (Fiscus, 1975) of Na, Cl, and SO_4 were calculated based on the total ion uptake, total transpiration, and concentration of the treatment solution (C_e):

$$\sigma = 1-[((m_{t2} - m_{t1})*W_{t2}) / (R_T*C_e)]$$

Where m_{t1} represents the average whole-plant ion concentration at the beginning of the experiment, m_{t2} represents the ion concentration of the whole plant after 3 weeks of treatment, W_{t2} represents the dry weight of the plant, and R_T represents the total transpiration over the 3 weeks of treatment. Negative values of σ indicate active transport, while values greater than 1 indicate efflux of the ion.

7.2.4 Data analysis

Data were analyzed using a general linear model (GLM). Means of treatments were compared by Duncans' multiple range test at each harvest date. Relationships between water relations parameters were examined using linear regression across all treatments. Cumulative transpiration rates were analyzed using GLM repeated measures.

7.3 Results

7.3.1 Water relations

By the end of the 3-week treatment period, root and shoot fresh weights were significantly reduced only by treatment with 60 mM Na_2SO_4 (Fig. 7.3.1.1). In this treatment fresh biomass accumulation rate was negative, indicating a loss of mass in these seedlings. No interaction was found between treatment and time for water potential, root respiration, and root hydraulic conductivity, and data were combined for further analysis. Shoot water potential (ψ) in all treated seedlings was less negative than in controls, with this difference being significant in seedlings treated with 120 mM NaCl (Table 7.3.1). Root respiration was also significantly reduced only in plants treated with 120 mM NaCl. Root hydraulic conductivity (Lpr) was reduced in all treatments, and this reduction was significant in the 60 mM NaCl + 30 mM Na_2SO_4 treatment. The rate of water uptake was reduced in all treatments. Daytime transpiration was also reduced by all salt treatments, with the greatest reductions found in plants treated with 120 mM NaCl (Fig. 7.3.1.2a). Transpiration rates measured at 1 week and 3 weeks did not significantly differ. Stomatal conductance was also reduced by all salt treatments, and did not differ between harvest dates (Fig. 7.3.1.2b). In the 60 mM NaCl and mixed salt treatments, stomatal conductance was reduced to approximately 30% of that measured in control seedlings, while conductance in the 120 mM NaCl and 60 mM Na_2SO_4 was significantly lower, at only around 10% that of controls. No relationships were found between Lpr and transpiration ($r = -0.02$) or transpiration and shoot water potential ($r = 0.015$). A

small but significant (P = 0.01) relationship was found between Lpr and shoot water potential (r = 0.289).

7.3.2 Ion uptake

In treatments with equivalent levels of Na, accumulation of Na in shoot tissue was greater in plants treated with 120 mM NaCl and mixed salt than in those treated with 60 mM Na_2SO_4 (Fig. 7.3.2.1a). Sodium concentrations were greater in shoots than in roots in seedlings treated with 120 mM NaCl, while the reverse was found for those treated with Na_2SO_4 or mixed salt. Chloride concentrations were greater in shoots than in roots for all treated plants (Fig. 7.3.2.1b). Shoot Cl appeared to be directly related to treatment Cl concentration, while root Cl concentrations were lower in plants treated with mixed salt than those in NaCl treatments. Root Cl concentrations differed little between plants treated with 60 mM NaCl and those treated with 120 mM NaCl. Roots accumulated similar levels of SO_4 in both treatments containing SO_4, while shoot accumulation was related to treatment concentration (Fig. 7.3.2.1c).

Root K was reduced to the same extent by the three high-Na treatments, although this reduction was statistically significant only in plants treated with 60 mM Na_2SO_4 (Fig. 7.3.2.2a). Shoot K concentrations were not affected by salt treatment. As seen for K, root Ca and Mg concentrations were reduced by the three high-salt treatments, while shoots concentrations did not differ significantly from controls (Fig. 7.3.2.2 b and c). For all three of these ions, shoot concentrations in treated plants were lower than concentrations at the beginning of the treatment period.

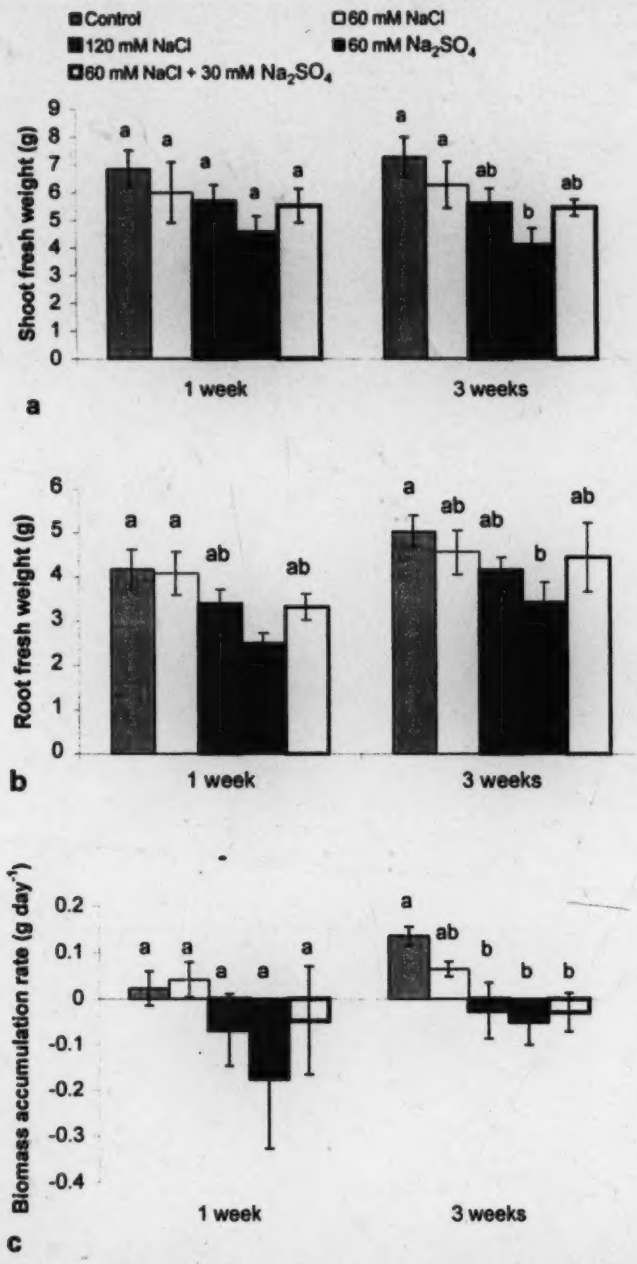

Fig. 7.3.1.1. Shoot fresh weight (a), root fresh weight (b), and biomass accumulation rate (c) of jack pine seedlings treated for one or three weeks with salt solution. Bars represent standard errors, and different letters indicate significant differences at α = 0.05 between treatments at a given harvest date.

Table 7.3.1. Osmotic potential of the treatment solution (treatment ψs), shoot water potential (ψ), difference in water potential between shoot and treatment solution (Δψ), water uptake per g fresh root weight calculated from total transpiration, root hydraulic conductance (Jv), and root respiration of jack pine treated with salt solution. Data from harvests at 1 week and at 3 weeks are combined. Standard errors are shown in parentheses. Different letters represent significant differences between treatments at α = 0.05.

	Treatment ψs (MPa)	Shoot ψ (MPa)	Δψ (MPa)	Water uptake (ml hr^{-1} g^{-1})	Jv x 10^{-7} (m^3 g^{-1} root FW s^{-1} MPa^{-1})	Root Respiration (mmol O_2 kg^{-1} min^{-1})
Control	.044	-1.04 (0.07) a	.996	0.081 (0.015)	3.78 (0.46) a	8.7 (0.47) a
60 mM NaCl	.341	-1.35 (0.20) a	1.009	0.031 (0.011)	2.63 (0.56) ab	8.2 (0.60) ab
120 mM NaCl	.639	-1.71 (0.22) b	1.071	0.045 (0.016)	2.89 (0.48) ab	7.1 (0.57) b
60 mM Na_2SO_4	.490	-1.2 (0.13) a	.710	0.044 (0.013)	2.87 (0.52) ab	8.7 (0.60) a
60 mM NaCl +30 mM Na_2SO_4	.564	-1.43 (0.16) ab	.866	0.050 (0.015)	1.91 (0.28) b	7.3 (0.35) ab

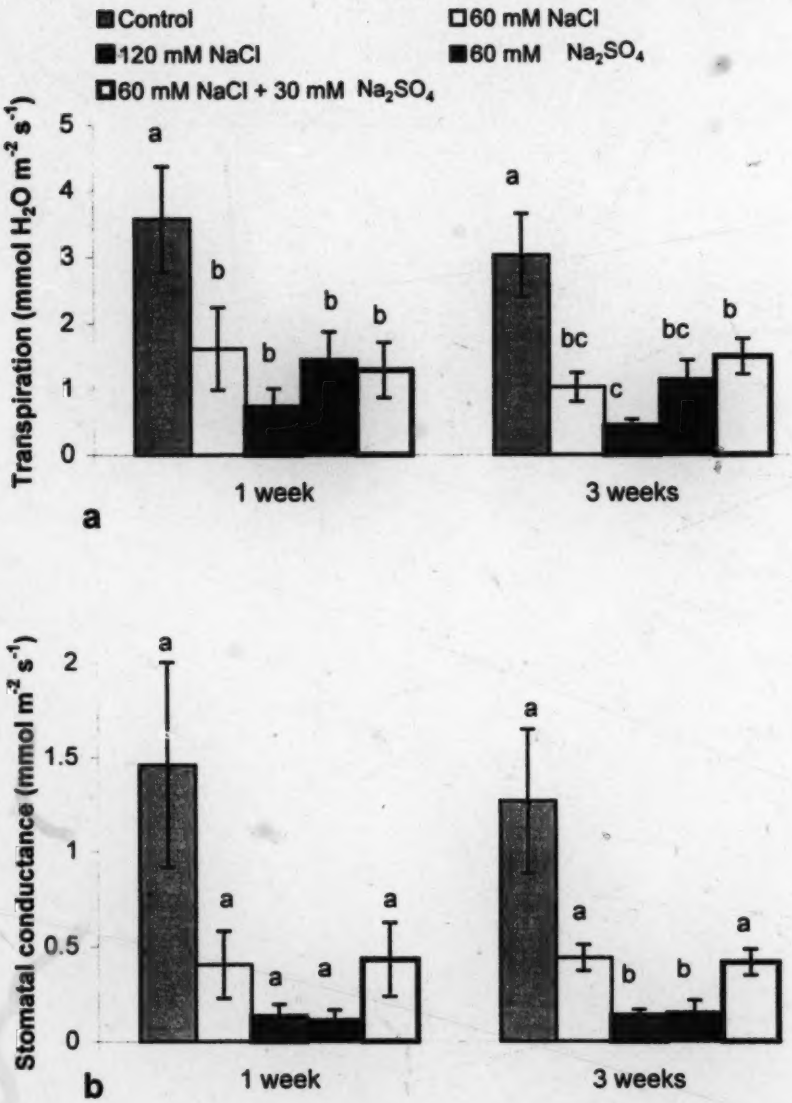

Fig. 7.3.1.2. Daytime transpiration rates (a) and stomatal conductance (b) of jack pine seedlings treated for 1week or for 3 weeks with salt solution. Bars represent standard errors, and different letters indicate significant differences at $\alpha = 0.05$ between treatments at a given harvest date.

Total transpiration was reduced by all salt treatments (Fig. 7.3.2.3). The total volume of water transpired per plant did not differ significantly between salt treatments over the 3-week treatment period. However, while transpiration of plants treated with 120 mM NaCl decreased slowly over the first 4 days in comparison with control plants, transpiration in plants receiving other salt treatments was reduced by approximately 50% over the first day of treatment. When expressed as a function of the volume of water transpired, root-to-shoot transport of Cl in the 120 mM NaCl treatment was more than double that of treatments containing 60 mM NaCl (Fig. 7.3.2.4 a). Sulfate transport was significantly greater in seedlings treated with 60 mM Na_2SO_4 than those treated with 30 mM Na_2SO_4 in the mixed salt treatment (Fig. 7.3.2.4 b). Sodium transport was greatest in plants treated with 120 mM NaCl, and least in those receiving 60 mM Na_2SO_4 (Fig. 7.3.2.4 c). Exclusion of Cl and Na from the root was more efficient at an external Cl concentration of 60 mM than at 120 mM (Table 7.3.2). Reflection coefficients show that root absorption of SO_4 is much lower than that of Cl. Roots showed a greater efficiency of Na exclusion in the mixed salt treatment than in the 60 mM NaCl treatment with a lower Na concentration.

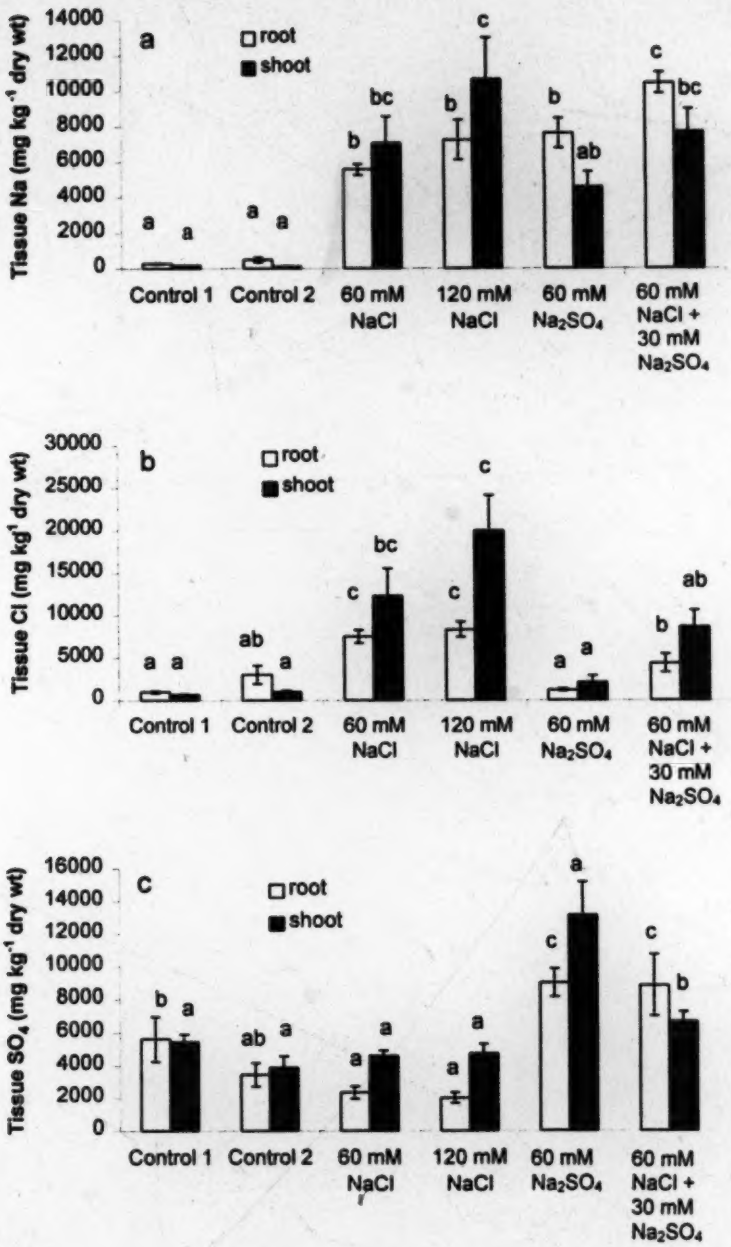

Fig. 7.3.2.1. Root and shoot concentrations of Na (a), Cl (b), and SO_4 (c) in jack pine seedlings at the beginning of the treatment period (Control 1) and after 3 weeks of treatment with control (Control 2) or salt solution. Bars represent standard errors, and different letters indicate significant differences between treatments at $\alpha = 0.05$.

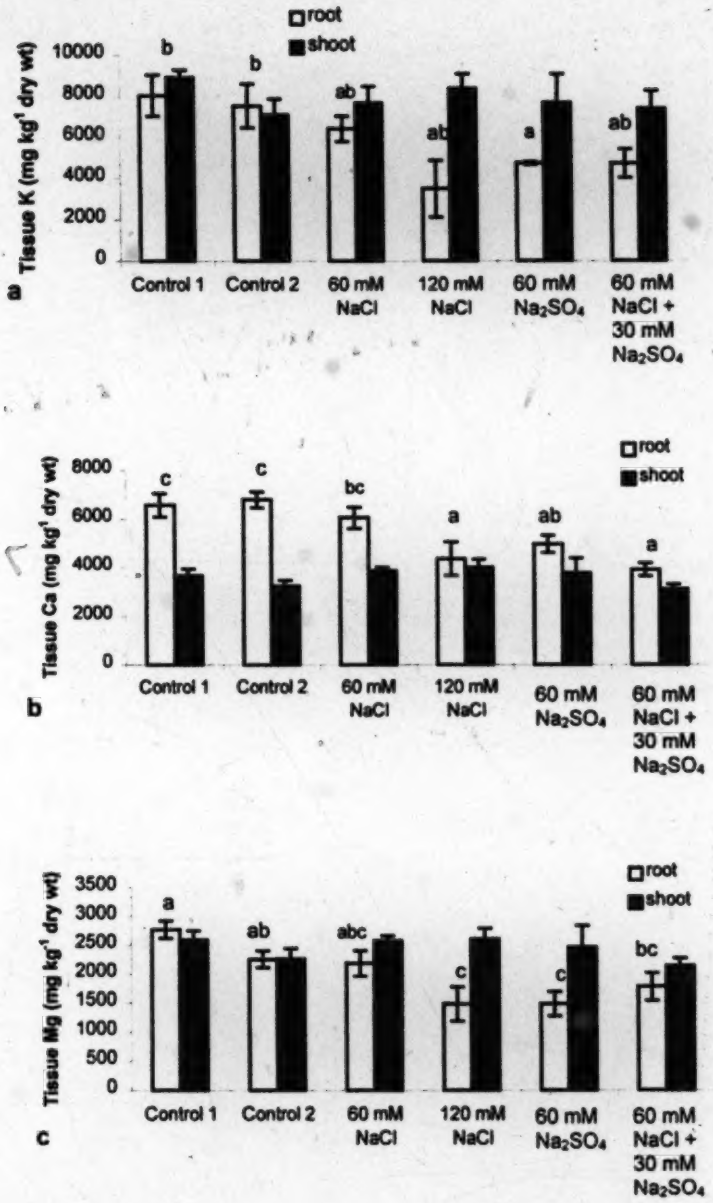

Fig. 7.3.2.2. Root and shoot concentrations of K (a), Ca (b), and Mg (c) in jack pine seedlings at the beginning of the treatment period (Control 1) and after 3 weeks of treatment with control (Control 2) or salt solution. Bars represent standard errors, and different letters indicate significant differences between treatments at $\alpha = 0.05$.

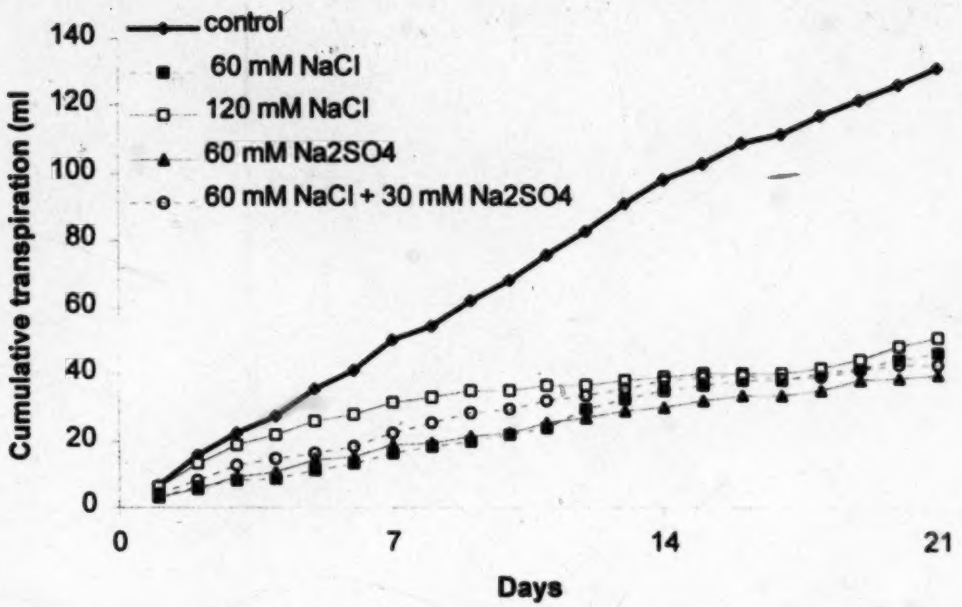

Fig. 7.3.2.3. Average cumulative transpiration per plant over a 3-week treatment period, of jack pine treated with salt solution.

Table 7.3.2. Reflection coefficients (σ) for Cl, Na, and SO_4 in jack pine treated for 3 weeks with salt solution.

	Cl	SO_4	Na
control	-0.322	1.130	
60 mM NaCl	0.798	1.381	0.811
120 mM NaCl	0.713	1.520	0.768
60 mM Na_2SO_4	-0.940	0.912	0.925
60 mM NaCl + 30 mM Na_2SO_4	0.847	0.989	0.884

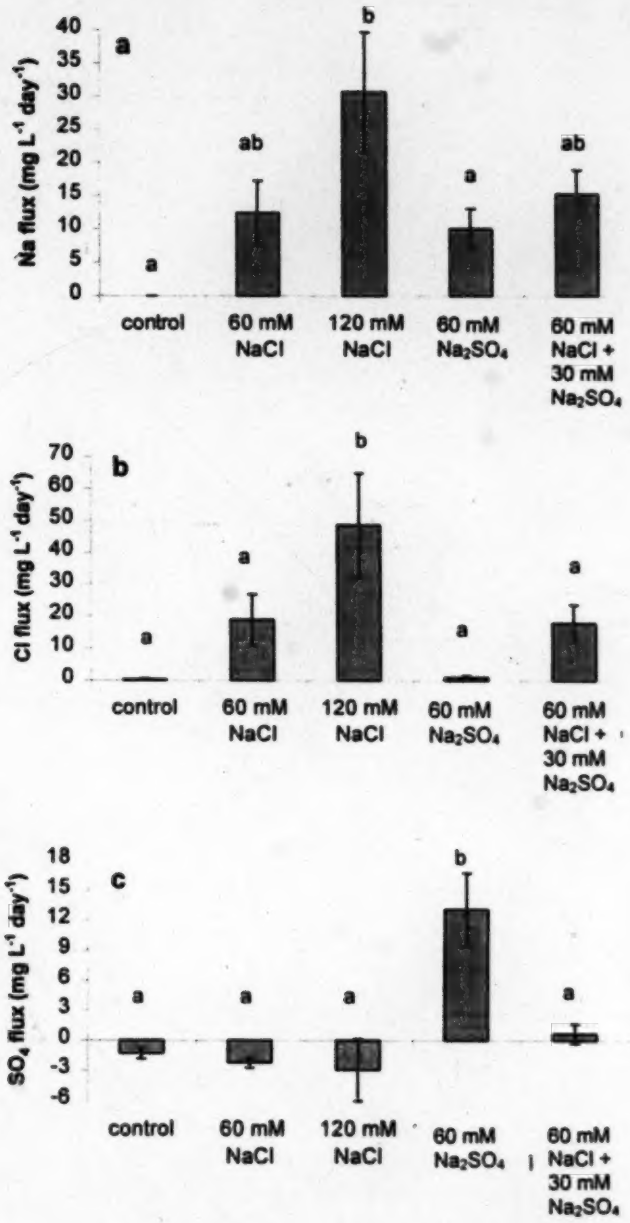

Fig. 7.3.2.4. Root-to-shoot movement Na (a), Cl (b), and SO_4 (c) in jack pine seedlings over 3 weeks of treatment with salt solution. Bars represent standard errors, and different letters indicate significant differences between treatments at $\alpha = 0.05$.

7.4 Discussion

Sodium chloride is the salt most commonly used to examine the mechanisms of salt tolerance in plants. Although NaCl is often used to model plant response to salinity, many saline soils contain SO_4 as the dominant or co-dominant anion. Na_2SO_4 and NaCl have been found to have very different effects on plant growth, pigment concentration, protein metabolism, cellular structure (Strogonov, 1973), nutrient interactions (Grattan and Grieve, 1999), and water relations (Redfield, 2000; Renault et al., 2001).

Results of NaCl treatments were relatively straightforward, and in agreement with the majority of previous reports. Transpiration and stomatal conductance declined with increasing salinity of the treatment solution, and were accompanied by a reduction in growth (Fig. 7.3.1.1), although this reduction was small due to the relatively short duration of the experiment. Reduced transpiration or stomatal conductance has also been reported in NaCl-treated olive (Tattini et al., 1995) and radiata pine (*Pinus radiata* D. Don) (Sands and Clarke, 1977; Myers et al., 1998). Plants treated with NaCl showed no visible wilting, and the water potential gradient from treatment solution to shoot was similar to that of control plants suggesting that osmotic adjustment had occurred, likely due to the accumulation of Na and Cl. In NaCl-treated radiata pine, a decrease in water potential was explained by the increase in inorganic ions in the shoot, and proline contributed little to the total osmotic adjustment (Sands and Clarke, 1977). Inorganic ions contributed more to total osmotic adjustment than organic ions in cotton (*Gossypium hirsutum* L.) (Zvi and Federman, 1991) and in corn roots (*Zea Mays* L) (Rodriguez et al., 1997). In contrast to celery (*Apium graveolens* L.), where transpiration rates and hydraulic conductance recovered slowly after the first week of NaCl treatment, apparently the result of osmotic adjustment, (Pardossi et al, 1998), no recovery of transpiration was apparent in radiata pine (Sands and Clarke, 1977) or in jack pine during the treatment period of my experiment.

Both shoot and root fresh weights were significantly reduced by treatment with 60 mM Na_2SO_4, where a large negative value for biomass accumulation was recorded over the first week of treatment. Dry weight was also lower in sorghum (*Sorghum bicolor* L.)

(Khan et al., 1995) and in red-osier dogwood seedlings (Renault et al., 2001) treated with Na_2SO_4 than with isomolar NaCl. In the current experiment, negative values of biomass accumulation and visual observations suggest that lower fresh weights in 60 mM Na_2SO_4 as compared with controls may have been due to a loss of necrotic roots to the treatment solution, and reductions in water content. Although water content was not measured in this experiment, some plants treated with Na_2SO_4 had visibly wilted and drying needles. Stomatal conductance in this treatment was lower than in isomolar NaCl, neither daytime transpiration rates (Fig. 7.3.1.2) or total transpiration (Fig. 7.3.2.3) differed between these treatments, and so was not likely the direct cause of growth reduction. In dogwood, a greater reduction in stomatal conductance of plants treated with Na_2SO_4 than with isomolar NaCl could be explained by the lower osmotic potential of the former, but the osmotic effect did not explain the lower growth rates and tissue water contents of plants treated with Na_2SO_4 (Renault et al., 2001). In contrast to jack pine treated with NaCl, I found that the water potential gradient was not maintained in Na_2SO_4-treated plants (Table 7.3.1).

In pepper plants (*Capsicum annuum* L.), an inverse relationship was found between transpiration and the osmotic potential of the nutrient solution, the water potential gradient from solution to leaf, and the internal resistance to water flow (Janes, 1970). In this experiment, increasing concentrations of nutrients were used to induce osmotic stress, and so effects may differ from plants treated with Na salts. Water flux into roots of tomato (*Lycopersicon esculentum* Mill.) and sunflower (*Helianthus annuus* L.) treated with NaCl was linearly related to the water potential difference between root exterior and leaves (Shalihevet et al., 1976), although this relationship may actually be non-linear across a wide range of osmotic pressures (Dalton et al, 1975). In the present study, transpiration and stomatal conductance of jack pine did not appear to be related to the osmotic potential of the treatment solution or to the difference in water potential across the root. Root hydraulic conductance (Jv) was reduced by all salt treatments, with a statistically significant reduction found in the mixed salt treatment which was also the treatment with the lowest osmotic potential. Interestingly, plants in this treatment showed a slightly higher rate of water uptake than plants in other salt treatments, and no correlation was found between transpiration rates and Jv. Root hydraulic conductivity of

bean (*Phaseolus vulgaris* L.), measured by pressurizing the root system to produce water influx, was greatly reduced by NaCl treatment (O'Leary, 1969). In corn roots, both water influx and efflux rates were reduced by the addition of NaCl to the growth medium (Evlagon et al., 1992). A systematic error may have occurred in my Lpr measurements as a result of the method used. The roots systems, immersed in treatment solutions, were subjected to pressure using deionized water. Therefore, an osmotic force driving water efflux was measured in addition to the hydrostatic force. The effects of osmotic forces on efflux have been modeled (Fiscus, 1975) and demonstrated in practice (O'Leary, 1969). The resulting error would be related to the osmotic potential of the treatment solution, and the actual reduction in Lpr could be somewhat greater than is shown by my data, particularly for the 120 mM NaCl and mixed salt treatments. This effect is likely minimal, however, as water flow resulting from hydrostatic forces appears to be greater than that from osmotic forces (Steudle, 1995).

Sodium accumulated in shoots to a greater extent in plants treated with NaCl than in those treated with Na_2SO_4 at isomolar Na levels. Shoot Cl and SO_4 concentrations appeared to be related to concentration of the treatment solution, and shoot concentrations of K, Ca, and Mg were little affected, although concentrations of these ions decreased roots of plants in high-Na treatments. Nutrient uptake in solution culture may be very different from field conditions because of different nutrient ratios and root morphology (Grattan and Grieve, 1999). My results however, are very similar to results obtained in sand culture using the same treatments (Chapter VI). Although three treatments had the same concentration of Na in the treatment solutions (120 mM NaCl, 60 mM Na_2SO_4, and 60 mM NaCl + 30 mM Na_2SO_4), the 120 mM NaCl produced more, and earlier visible injury, significantly lower shoot water potential and root respiration. These effects are likely the result of high concentrations of Na and Cl, and low K in tissue. At an external concentration of 120 mM NaCl, rates of Na and Cl transport to the shoot were high, indicating high concentrations of these ions in the xylem sap. While similar transpiration rates were recorded in seedlings of all salt treatments, ion flux and reflection coefficients differed greatly indicating that neither root-to-shoot transport of ions nor uptake rate was directly dependant on transpiration rates (Fig. 7.3.2.2). These results are in agreement with those of Meiri et al. (1970), who also failed to find a

relationship between transpiration and ion uptake in bean. The high uptake rates reflect not only the concentration of the treatment solution, but also a reduction in the ability of the root to restrict uptake, as seen by the lower reflection coefficients. There is a possibility that reductions in the reflection coefficients could be due to increases in the proportion of apoplastic flow. Reductions in hydraulic conductivity in corn roots treated with NaCl were found to result from reduced cell-to-cell water transport across the root (Azaizeh et al, 1992), and an increase in the proportion of apoplastic flow has been shown to contribute significantly to Na uptake (Yeo et al., 1987). The current composite transport model of root water flow predicts that as root hydraulic conductivity decreases, the contribution of apoplastic flow to total water flow across the root increases (Steudle, 1995). However, this model does not appear to explain the observed differences in Na transport between plants treated with NaCl and Na_2SO_4. Although not statistically different from controls, similar reductions in Lpr were observed for 120 mM NaCl and 60 mM Na_2SO_4 treatments, but the NaCl-treated plants had a greater Na flux and lower reflection coefficient. The only treatment showing significantly reduced Lpr (60 mM NaCl + 30 mM Na_2SO_4) had a relatively high Na reflection coefficient. Reductions in membrane selectivity also be the result of Cl toxicity, as suggested by previous studies (Chapter 6). Reduced root respiration could conceivably contribute to increased ion uptake, although the outward Na flux was found not to be metabolically dependant (Jacoby, 1979). While the mechanisms of Na and Cl transport are independent and not directly coupled (Maas and Ogata, 1972; Grieve and Walker, 1983), my data shows that the uptake and transport of Na is dependant more upon external Cl levels than on external Na levels.

In summary, treatment with NaCl resulted in uptake and accumulation of Na and Cl ions in the roots and shoots, reduced root respiration, and shoot water potential. Seedlings treated with NaCl maintained the water potential gradient across the root. In contrast, Na_2SO_4 treatment resulted in a reduction in the water potential gradient, root respiration rates similar to control plants, and lower rates of Na accumulation in shoot tissue. Transpiration was similarly reduced by all salt treatments, and was not responsible for differences in Na flux between NaCl and Na_2SO_4-treated plants. The greater uptake of Na in NaCl-treated plants appears to be the result of a greater

permeability of the root to Na, as shown by the reflection coefficients. Plants receiving the mixed-salt treatment had a greater ability to restrict the translocation of Cl and SO_4 to the shoot, and retained a greater greater concentration of Na in the roots, than plants treated with a single salt. These phenomena, which could be of great importance in naturally saline soils, are worthy of further study.

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Chapter VIII

Conclusions

This research grew from the needs of oil sands mining operations to understand how saline tailings materials may influence plant growth. This knowledge is needed to develop tailings management strategies for successful vegetative reclamation. Therefore, one of the main objectives of this research was to identify those principal components of CT water that may be injurious to jack pine (*Pinus banksiana* Lamb.). Plants treated with CT water showed significant needle necrosis, significant reductions in all measured growth parameters, and reduced chlorophyll a and carotenoid content (Chapter IV). Elemental analysis of shoots of CT-treated plants showed that plant nutrient status was affected. Reductions in shoot concentrations of the essential elements Fe and Mo are likely the result of the high pH of CT, as tissue concentrations of these elements were unaffected by salt treatments. Reductions in K are likely related to the high Na content of the CT water. While nutrient deficiency could potentially limit growth in the long term, it was not directly related to the injury seen in CT-treated plants. Rather, injury appears to be the result of ion toxicity, as elevated levels of Na and Cl were correlated with needle necrosis. B and Sr, although present in elevated levels in the shoot, were not directly related to injury. Further studies, therefore, focussed on the two main salts found in CT, NaCl and Na_2SO_4.

Jack pine responded very differently to NaCl and Na_2SO_4. Early investigations (Chapter IV) showed that NaCl was more injurious to jack pine compared with isomolar Na_2SO_4, and resulted in greater needle necrosis, reductions in carotenoid content, and delays in bud flushing. The results suggest that ion toxicity, rather than water deficit stress, was responsible for the observed injury. The relationships between growth and injury parameters and shoot elemental composition were tested, and showed that shoot Na concentration was related to needle necrosis in NaCl treated plants, but not in those treated with Na_2SO_4. In a similar study on ponderosa pine, Cl was the factor with the greatest correlation to needle necrosis in ponderosa pine, while Na was not found to be correlated with necrosis (Spotts et al. 1972). Spotts et al. (1972) analyzed data across

treatments of NaCl, Na_2SO_4, $CaCl_2$, and $MgCl_2$. This analytical approach could only demonstrate that Cl treatment resulted in both greater Cl uptake and greater injury compared with Na_2SO_4 treatment. In contrast, the data presented in the present dissertation have been analyzed within treatments (Table 5.3.2), and this resulted in a different conclusion; that needle Na concentration is related to injury, and that this occurs only in the presence of Cl.

In comparing the effects of different salts, interpretation may be difficult as the factors of anion concentration, cation concentration, and osmotic potential may be confounded with salt type. Such was the case in a study that compared membrane leakage in soybean treated with iso-osmotic NaCl and Na_2SO_4 (Leopold and Willing, 1984). Although a greater leakage of organic solutes was measured in NaCl-treated plants, the results could not be attributed to ion toxicity because treatment solutions contained different ion concentrations. The studies presented in Chapters VI and VII focussed on ion toxicity, and treatment concentrations were selected to allow direct comparisons between levels of Na and Cl. My results clearly show a relationship between electrolyte leakage and Cl concentration of the treatment solution. Although membrane injury could potentially result from either Na or Cl, as the accumulation of these ions in the tissue appears to be closely linked, results of electrolyte leakage tests and selection coefficients are evidence that Cl is a factor in membrane injury.

The uptake and transport of Na to the shoot also appears to depend on the presence of Cl, rather than the treatment concentration of Na. This increase in Na transport was not simply related to higher transpiration rates, but is an actual difference in Na flux, with the roots showing less ability to restrict Na uptake in the presence of Cl, observed as lower Na reflection coefficients. These results are consistent with the hypothesis of Cl-induced membrane leakage. Reflection coefficients and ion uptake as a function of transpiration have previously been reported for NaCl treated sunflower (*Helianthus annus* L.) and tomato (*Lycopersicon esculentum* Mill.) (Shalhevet et al., 1976). However, in those studies these parameters were calculated based on daytime transpiration rates and estimates of root area. The method used in Chapter VII was designed to accurately measure total water uptake by the plant. Because initial tissue concentrations of Na and Cl were very low in comparison to treated plants, estimates of

total ion uptake also have a high degree of accuracy. The reflection coefficients and ion flux presented here should therefore be very close to actual values, and show differences between plants treated with NaCl and Na_2SO_4 that have not been previously reported.

A number of mechanisms exist by which plants may tolerate salinity, and this research has provided evidence of some of these mechanisms in jack pine. My studies show that jack pine is able to sequester both Na and Cl in the root. At high levels of Na and Cl in the substrate, such as the levels used in these studies, this mechanism was overcome after approximately one week of exposure, and translocation of these elements to the shoot increased. This increase in translocation could occur when root tissue reaches a saturation point, and is no longer able to sequester Na and Cl in the vacuole. At lower salinity levels, this mechanism may be sufficient and allow the plant to maintain growth while accumulating minimal amounts of these ions in the shoot tissue. Even at the high salt levels used in these experiments, the roots system is an effective barrier to the passage of ions to the shoot. Reflection coefficients show that the uptake of Cl is highly regulated, with negative coefficients indicating active uptake under low levels of Cl in the root environment. At high ambient Cl levels in solution, the root system is able to prevent the entry of over 70% of the Cl that would flow passively into the plant with the transpiration stream. Within shoot tissue, potentially toxic ions may be sequestered in the vacuole. Concentrations of Na and Cl in new needles showing no visible injury exceeded concentrations in necrotic needles. Such differences in the ability of tissues to tolerate salts are likely due to differences in the ability to compartmentalize these ions, and thereby maintain low cytoplasmic concentrations (Yeo, 1998).

Another potential mechanism by which plants could survive periods of high salinity would be to reduce transpiration rates, thereby theoretically reducing the translocation of ions to the shoot. Results of this research (Chapter VII), however, show that transpiration rates are not related to the translocation rate of salt ions. Higher salt concentrations in the substrate resulted in greater translocation to the shoot, although transpiration was similarly reduced in all treatment concentrations. Neither was transpiration rate related to salt accumulation in shoots of different individuals exposed to the same treatment solution. These results bring into question the importance of transpiration in ion transport as has been recently proposed by Dalton et al. (2000).

While some movement of the transpiration stream is undoubtedly required to deliver ions to the shoot, I found that the concentration of ions in the transpiration stream do not necessarily reflect the concentration in the treatment solution, indicating that entry of ions to the xylem is regulated by processes independent of the bulk flow of water. Entry of ions to the xylem is dependant on membrane selectivity, and this selectivity is affected by the presence of Cl.

The maintenance of a high K:Na ratio has been associated with salt tolerance in some species (Iyengar and Reddy, 1994), likely due to a greater degree of K/Na selectivity. Jack pine appears to have little ability to selectively take up K, in the presence of high external Na concentrations. Roots of treated plants showed K concentrations to be reduced by more than half, and after one and three weeks in NaCl treatment, both uptake and root-to-shoot transport rates were negative, indicating a net loss of K from the plant. But although these plants were unable to maintain uptake of K, concentrations of K in new tissue was more than double that found in older needles of plants treated with 60 mM NaCl, indicating that jack pine is able to selectively transport K into these tissues.

The apparent importance of Cl has implications both in practice, and theory. This research suggests that reclamation planners should monitor Cl levels on reclamation sites, and avoid the placement of jack pine on sites with high Cl levels. Such sites could be planted with Cl tolerant species to facilitate the leaching of Cl prior to planting jack pine. This research also suggests that because jack pine appears to be able to sequester both Na and Cl in the roots, salt exposure of short duration may not result in injury. Na and Cl were found to be the primary factors contributing to injury in CT water, but results of salt-treated plants suggest that this may not hold true in CT water with a lower Cl content. Nutrient deficiency in CT-treated plants, while not likely inhibitory in this short-term study, could potentially limit growth in the long term. Results of the pre-treatment experiment in Chapter III show that pre-treatment with NaCl has a potential for increasing the survival rates of nursery-grown seedlings planted on reclamation sites.

The possible toxicity of the Cl ion has received relatively little attention in recent salinity studies, although NaCl has been the predominant salt used in research into the mechanisms of salt tolerance. Research into increasing salt tolerance by genetically

increasing compartmentation of Na (Apse et al., 1999) failed to consider transport of Cl, which must also be accumulated in the vacuole as a counter ion (Frommer et al., 1999). The interaction between Na and Cl may prove to be important in attempts to increase the salt tolerance of some species, or when trying to relate NaCl effects in laboratory or greenhouse studies to field conditions where Cl might not be the predominant ion.

A high individual variation in jack pine response to salt was found, as has been reported for other coniferous species, but seed sources did not significantly differ in salt tolerance. Although not statistically significant, some interesting differences were observed between seed sources. Plants from the Smoky Lake seed source appeared to accumulate more Na in roots and shoots in Na_2SO_4 treatments than in NaCl treatments, in contrast to the other two seed sources (Fig. A 3.3.1.2). These data suggest that further study of the genetic differences in jack pine salt tolerance is worthwhile. Individuals showed a high degree of variation in the uptake of potentially toxic Na and Cl. Potential sources of variation include differences in the ability to compartmentalize Na and Cl in the root cells, differences in the amount of apoplastic flow, and variation in the amount of membrane leakage induced by NaCl treatment.

Several factors should be considered in using the results of this study to predict jack pine response to salts under field conditions. These experiments were performed in a controlled environment chamber, under optimal environmental conditions, and with an adequate nutrient supply. Prior to this study, no information was available on the response of jack pine to salts, and so the intent of this research was to provide a fundamental investigation. The interactions between salinity and environmental factors or nutrient supply, are likely to be important, as indicated by the field study, but are beyond the scope of this research. The use of sand and solution culture also limits the extrapolation of these results to the field, although jack pine tends to grow on sandy sites, some of which have very low organic content in the soil. Finally, these experiments were relatively short in duration, with the longest salt exposures being 10 weeks. Although nutrient deficiency was not found to be a factor in the short-term, an alteration of nutrient status was observed, and may limit growth in the long-term.

8.1 Literature cited

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Appendix A

Methodology

Carotenoid analysis

Approximately 0.500 g of freeze-dried needle tissue was weighed, and the weights were recorded. Tissue was ground with a mortar and pestle in successive changes of 85% acetone, until colorless. The volume of acetone was made up to 10 ml, and the total carotenoid concentration of the extract was determined by spectrophotometer. Carotenoid concentration was then calculated based on the dry weight of the sample, using the formula of Davies (Davies, B. H., 1976, Carotenoids. *In* Chemistry and Biochemistry of Plant Pigments vol. 2, T. W. Goodwin *ed.* Academic Press, New York. Pp. 38 – 155) which corrects for the interference of chlorophyll in the solution:

$$\text{total carotenoids} = 1\ A_{480} + 0.114\ A_{663} - 0.638\ A_{638}$$

Where A_x is the absorbance at $\lambda = x$.

Chlorophyll analysis

The method described above for determination of carotenoid content is also commonly used for determining chlorophyll concentration. However, a small amount of pigment remains on the mortar and pestle which could potentially result in appreciable error when using small amounts of tissue. Determination of chlorophyll concentrations in one-month-old seedlings therefore required the development of a method by which very small quantities of tissue could be extracted and accurately assessed. Freeze-dried needle tissue was weighed to 0.0200 ± 0.0002 g and placed into a 20 ml glass vial, to which 2 ml of 100% methanol was added. The vial was capped, covered to prevent degradation of pigments by light, and placed on an orbital shaker. After 4 hours the solution was removed and reserved, and 2 ml of fresh methanol was added. After a second 4-hour period the solution was again removed and 1 ml methanol was added. After a total extraction period of 24 hours, the 3 portions of solution containing the

extracted pigments were combined, and topped up to a volume of 5.0 ml as a small amount of methanol was lost to evaporation. The concentration of chlorophylls a and b in this solution was then determined using a spectrophotometer, and calculated using the formulas of MacKinney (Sestak, K., J. Catsky, and P. G. Jarvis, 1971, Plant photosynthetic production. W Junk NV, The Hague):

$$\text{Chl a} = 6.58\ A_{666}$$
$$\text{Chl b} = 3.55\ A_{653}$$

The completeness of this extraction method was tested by extracting the tissue again with either methanol or with 85% acetone. These extracts were found to contain less than 3% of the amount of pigments previously removed by the methanol extraction process.

Determination of Cl and SO_4 concentration in tissue

Chloride electrode method for determination of tissue Cl

Freeze-dried needle tissue was finely ground in liquid N using a mortar and pestle. The sample, weighed to 0.100 ± 0.005 g, was added to 10 ml of 0.5 M HNO_3 and shaken for 30 min. on an orbital shaker. Standard solutions from 10 to 1000 ppm Cl were used to determine the slope of the regression line using the chloride selective electrode (Accumet, Fisher Scientific, Edmonton, AB). This standardization was repeated if $r^2 < 0.98$, and after every 3 hours of use. 200 mM of 5 M $NaNO_3$ was then added to the sample, which was placed on a magnetic stirrer, and a reading taken after the electrode was allowed to equilibrate for 4 min. The Cl concentration of the tissue was then calculated using the regression equation and the weight of the tissue.

Hot water extraction of tissue for determination of Cl and SO_4 by ion chromatography

Freeze-dried samples were ground in liquid N. A weighed amount of tissue (0.050 ± 0.01 g) was placed into a 15 ml polystyrene centrifuge tube, to which was added 5.0 ml of near-boiling water. The tube was capped, shaken for 30 min., then centrifuged at 2000 rpm for 20 min. The solution was poured off and reserved. A second 5 ml of hot water was added to the remaining residue, and the process was repeated. The two portions of solution were combined and filtered through a 45 micron syringe filter. Concentrations of Cl and SO_4 in this extract were determined by ion chromatography, by technicians at Syncrude Canada.

Tests of ion concentrations in 3 subsequent portions of extract showed that for levels of tissue Cl above 10,000 mg g^{-1} DW over 95% of extractable Cl was removed by the first two extractions (Fig. A1). At tissue Cl levels below 600 mg g^{-1} DW, approximately 80% of Cl was removed by the first two extractions. Approximately 92% of extractable sulfate was removed by the first extraction, and the remainder by the second extraction.

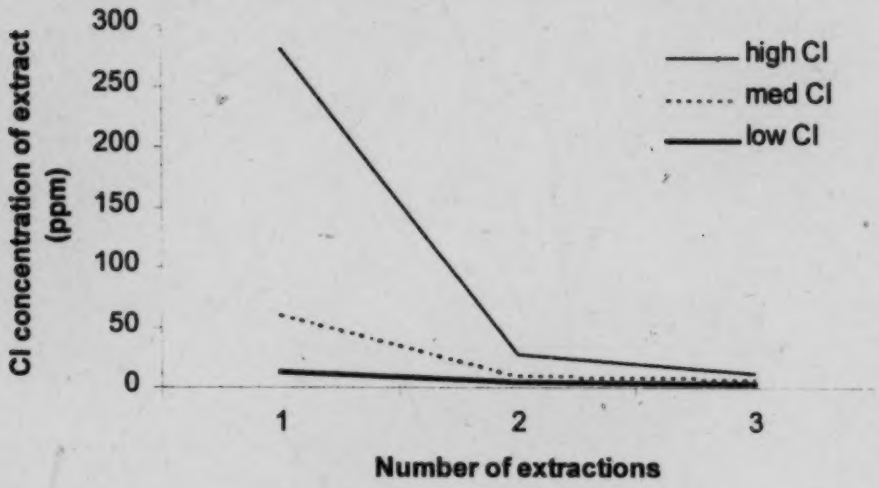

Fig. A1. Concentration of Cl in three successive hot-water extracts of root or needle tissue.

Determination of Na, K, and N concentrations by atomic absorption

Freeze-dried tissue was ground in liquid N for analysis of Na and K. Tissue was weighed to 0.050 ± 0.005g and placed in glass digestion tubes to which 5 ml of concentrated H_2SO_4, and 1 ml H_2O_2 were added. Digestion tubes were placed in a ceramic block heater at 360°C for 30 min. Tubes were removed, allowed to cool, and the process was repeated using 2 ml of H_2SO_4, and 1 ml H_2O_2. The extracted sample was diluted to a volume of 50 ml, and the Na and K concentration of the extract was measured using atomic absorption by technicians at the University of Alberta Department of Renewable Resources.

Determination of elemental composition by ICP-OES

Freeze-dried tissue was ground in liquid N. Tissue was weighed to 0.025 ± 0.005g and placed into a polyethylene microwave digestion vessel to which 5 ml concentrated HNO_3 was added. The vessel was sealed and heated in a microwave for the following times series at the given microwave powers: 1 min. at 50%, 1 min. at 0%, 1 min. at 50%, 2 min. at 0%, 1 min. at 75%, 1 min. at 0%, 1 min. at 75%, 2 min. at 0%, 1 min at 100%, 2 min. at 0%, and 1 min. at 100%. Vessels were rinsed with deionized water, and additional water was added to bring the volume up to 100 ml. The solution was filtered through a 45 micron syringe filter. Concentrations of B, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Sr, Sn, and Zn in this extract were determined by ICP-OES, by technicians at Syncrude Canada. Concentrations of Al, Cd, Cr, Ni and Pb were below the detection limits of the instrument.

Table A1. Chemical quantities used in nutrient mix during plant maintenance and experimental treatment periods (maintenance), and during the induction of dormancy (dormancy).

	Maintenance ($mg\ L^{-1}$)	Dormancy ($mg\ L^{-1}$)
KNO_3	72	0
KH_2PO_4	264	395
$Ca(NO_3)_2$	590	295
$CaCl_2$	0	183
$MgSO_4$	608	608
FeEDTA	19.4	19.4
$MnCl_2$	1.44	1.44
H_3BO_3	1.43	1.43
$ZnSO_4$	0.61	0.61
$CuSO_4$	1.96	1.96

Appendix B

Supplementary data and analyses

One-month-old seedlings treated for 14 days (pre-treatment) (Chapter III)

Model: $Yijk = \mu + \gamma_i + \rho_j + (\gamma\rho)_{ij} + \delta_k(\gamma_l) + \varepsilon_{ijk}$

Where γ_i represents pre-treatment (fixed factor), ρ_j represents seed source (fixed factor) $(\gamma\rho)_{ij}$ represents pre-treatment by seed source interaction, and δ_k represents replicate nested within pre-treatment (random factor).

Table A2. ANOVA for stem length of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
SS	2	0.733	1.265	0.284
PT	5	4.623	7.978	< 0.001
REP	2	2.748	4.741	0.009
SS x PT	10	0.549	0.948	0.489
Error	314	0.580		

Table A3. Mean (± SE) stem length (cm). Combined data from 3 seed sources. Different letters represent significant differences between pre-treatments at $\alpha = 0.05$.

Treatment	Mean (cm)
Control	5.72 (0.09) c
30 mM Na_2SO_4	5.41 (0.12) b
60 mM Na_2SO_4	5.05 (0.13) a
30 mM NaCl	5.77 (0.10) c
60 mM NaCl	5.32 (0.10) ab
CT water	5.11 (0.08) a

Table A4. ANOVA for total chlorophyll (a+b) in needles of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
SS	2	55.186	11.415	< 0.001
PT	5	35.384	7.319	< 0.001
REP	2	5.328	1.102	0.333
SS x PT	10	5.715	1.182	0.302
Error	310	4.834		

Table A5. ANOVA for chlorophyll a:b ratio in needles of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
SS	2	1.968	1.382	0.253
PT	5	1.985	1.394	0.226
REP	2	3.516	2.469	0.086
SS x PT	10	1.329	0.934	0.502
Error	309	1.424		

Table A6. Mean (± SE) chlorophyll concentration of needles from three seed sources. Combined data from all pre-treatments. Different letters represent significant differences between seed sources sources at $\alpha = 0.05$.

Seed source	Mean (mg g^{-1} FW)	
Syncrude	11.16	(0.22) b
Suncor	10.27	(0.25) a
Smoky Lake	9.74	(0.17) a

Table A7. Mean (± SE) chlorophyll concentration of needles from six pre-treatments. Combined data from three seed sources. Different letters represent significant differences between pre-treatments at $\alpha = 0.05$.

Treatment	Mean (mg g^{-1} FW)
Control	10.37 (0.32) b
30 mM Na_2SO_4	11.80 (0.33) c
60 mM Na_2SO_4	10.42 (0.37) b
30 mM NaCl	10.59 (0.25) b
60 mM NaCl	9.37 (0.31) a
CT water	10.10 (0.25) ab

Table A8. ANOVA for total dry weight of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
SS	2	< 0.0000	0.030	0.971
PT	5	0.0105	9.035	< 0.000
REP	2	0.0039	3.353	0.036
SS x PT	10	0.0018	1.563	0.117
Error	314	0.0012		

Table A9. ANOVA for shoot:root dry weight ratio of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
SS	2	2.154	0.615	0.542
PT	5	10.963	3.128	0.009
REP	2	4.522	1.290	0.277
SS x PT	10	4.214	1.202	0.289
Error	309	3.505		

Table A10. Mean (± SE) dry weight (DW) of seedlings (shoot and root combined), and shoot:root ratio based on dry weight. Combined data from 3 seed sources. Different letters represent significant differences between treatments at $\alpha = 0.05$.

Treatment	Mean DW (g)	Shoot:root
Control	0.124 (0.005) b	2.68 (0.29) ab
30 mM Na_2SO_4	0.093 (0.006) a	1.95 (0.11) a
60 mM Na_2SO_4	0.086 (0.005) a	2.75 (0.16) b
30 mM NaCl	0.114 (0.005) b	2.36 (0.07) ab
60 mM NaCl	0.095 (0.005) a	3.11 (0.40) b
CT water	0.090 (0.003) a	2.96 (0.20) b

Table A11. ANOVA for shoot water content (% FW) of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
SS	2	0.0103	6.102	0.003
PT	5	0.0091	5.415	< 0.001
REP	2	0.0071	4.214	0.016
SS x PT	10	0.0033	1.946	0.039
Error	309	0.0017		

Table A12. ANOVA for root water content of seedlings (% FW)from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
SS	2	0.00005	0.134	0.875
PT	5	0.00183	4.963	< 0.001
REP	2	0.00160	4.344	0.014
SS x PT	10	0.00061	1.641	0.094
Error	310	0.00037		

Table A13. Mean (± SE) water content (% FW) of shoots and roots from three seed sources. Combined data from all pre-treatments. Different letters represent significant differences between seed sources sources at $\alpha = 0.05$.

Seed source	Shoot water content (% FW)	Root water content (% FW)
Syncrude	0.730 (0.005) a	0.889 (0.002) a
Suncor	0.734 (0.004) a	0.890 (0.002) a
Smoky Lake	0.748 (0.002) b	0.890 (0.002) a

Table A14. Mean (± SE) water content (% FW) of shoots and roots from 6 salt pre-treatments. Combined data from 3 seed sources. Different letters represent significant differences between pre-treatments at $\alpha = 0.05$.

Treatment	Shoot water content (% FW)	Root water content (% FW)
Control	0.739 (0.003) a	0.884 (0.003) a
30 mM Na_2SO_4	0.750 (0.004) a	0.886 (0.002) ab
60 mM Na_2SO_4	0.713 (0.013) b	0.899 (0.003) c
30 mM NaCl	0.744 (0.004) a	0.884 (0.003) a
60 mM NaCl	0.739 (0.003) a	0.892 (0.002) bc
CT water	0.738 (0.003) a	0.892 (0.003) bc

Table A15. ANOVA for shoot Na concentration (mg kg^{-1} FW) of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
PT	5	17.641	14.597	0.000
SS	2	1.497	1.239	0.302
REP	2	0.713	0.590	0.560
SS x PT	10	1.212	1.003	0.461
Error	34	1.209		

Table A16. ANOVA for shoot K concentration (mg kg^{-1} FW) of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
PT	5	16.527	2.777	0.033
SS	2	1.528	0.257	0.775
REP	2	8.394	1.411	0.258
SS x PT	10	4.200	0.706	0.713
Error	34	5.950		

Table A17. ANOVA for root Na concentration (mg kg^{-1} FW) of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
PT	5	24.693	31.047	< 0.001
SS	2	0.231	0.291	0.749
REP	2	0.557	0.700	0.503
SS x PT	10	1.977	2.486	0.023
Error	34	0.795		

Table A18. ANOVA for root K concentration (mg kg^{-1} FW) of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
PT	5	20.409	1.686	0.165
SS	2	18.857	1.557	0.225
REP	2	35.093	2.898	0.069
SS x PT	10	9.597	0.793	0.636
Error	34	12.107		

Seven-month-old seedlings treated for 10 weeks: effects of pre-treatment (Chapter III)

Model: $Y_{ijkl} = \mu + \gamma_i + \rho_j + (\gamma\rho)_{ij} + \tau_k + (\gamma\tau)_{ik} + (\rho\tau)_{jk} + (\gamma\rho\tau)_{ijk} + \delta_l + \varepsilon_{ijkl}$

Where γ_i represents pre-treatment (fixed factor), ρ_j represents seed source (fixed factor) $(\gamma\rho)_{ij}$ represents pre-treatment by seed source interaction, τ_k represents treatment, $(\gamma\tau)_{ik}$ represents pre-treatment by treatment interaction, $(\rho\tau)_{jk}$ represents seed source by treatment interaction, $(\gamma\rho\tau)_{ijk}$ represents the three-way interaction between seed source, pre-treatment and treatment, and δ_k represents block (random factor).

Table A19. ANOVA for the number of days required for flushing of the terminal bud of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, and 4 salt treatments (TRT) at 7-months-of-age, in 4 replicate blocks of treatment trays (REP).

Source	df	Mean square	F	p value
SS	2	458.06	9.22	< 0.001
PT	5	72.99	1.47	0.197
TRT	3	1146.16	23.08	< 0.001
REP	3	182.29	3.67	0.012
SS x PT	10	55.09	1.11	0.351
SS x TRT	6	46.74	0.94	0.464
PT x TRT	15	34.45	0.69	0.793
SS x PT x TRT	30	42.48	0.86	0.691
Error	1292	49.66		

Table A20. Mean number of days (± SE) required for bud flushing of three seed sources. Combined data from all pre-treatments and treatments. Different letters represent significant differences between seed sources.

Seed source	Mean (± SE)
Syncrude	17.4 (0.42) a
Suncor	19.1 (0.44) b
Smoky Lake	19.3 (0.45) b

Table A21. Mean number of days (± SE) required for bud flushing of seedlings treated for 10 weeks with CT water or salt solution. Combined data from seed sources and pre-treatments. Different letters represent significant differences between seed sources.

Treatment	Mean (± SE)
Control	16.7 (0.50) a
60 mM NaCl	21.5 (0.50) c
60 mM Na_2SO_4	17.5 (0.50) ab
CT water	18.6 (0.50) b

Table A22. ANOVA for total stem elongation (cm) over a 10-week treatment period of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, and 4 salt treatments (TRT) at 7-months-of-age, using 4 blocks of treatment trays (REP).

Source	df	Mean square	F	p value
SS	2	2.99	3.29	0.038
PT	5	2.62	2.87	0.014
TRT	3	159.87	175.62	< 0.001
REP	3	3.94	4.32	0.005
SS x PT	10	0.65	0.71	0.712
SS x TRT	6	1.37	1.50	0.174
PT x TRT	15	1.89	2.07	0.009
SS x PT x TRT	30	0.46	0.50	0.989
Error	1416	0.91		

Table A23. Mean (± SE) stem elongation (cm) over a 10-week treatment period of three seed sources. Combined data from all pre-treatments and treatments. Different letters represent significant differences between seed sources.

Seed source	Mean (cm)
Syncrude	1.40 (0.06) a
Suncor	1.58 (0.06) b
Smoky Lake	1.52 (0.06) ab

Table A24. Mean (± SE) stem elongation (cm) over a 10-week treatment period of seedlings treated for 10 weeks with CT water or salt solution. Combined data from seed sources and pre-treatments. Different letters represent significant differences between seed sources.

Treatment	Mean (cm)
Control	2.52 (0.07) c
60 mM NaCl	1.30 (0.07) b
60 mM Na_2SO_4	0.89 (0.07) a
CT water	1.33 (0.07) b

Table A25. Mean (± SE) stem elongation (cm) over a 10-week treatment period of seedlings treated for 10 weeks with CT water or salt solution. Combined data from seed sources. Different letters represent significant differences between pre-treatments within a treatment group.

	Treatment			
Pre-treatment	Control	60 mM NaCl	60 mM Na_2SO_4	CT water
Control	2.33 (0.14) a	1.14 (0.09) a	1.06 (0.06) b	1.34 (0.08) ab
30 mM Na_2SO_4	2.48 (0.14) a	1.25 (0.07) ab	0.76 (0.06) a	1.34 (0.08) ab
60 mM Na_2SO_4	2.52 (0.14) a	1.46 (0.10) b	0.91 (0.07) ab	1.07 (0.06) a
30 mM NaCl	2.76 (0.17) a	1.24 (0.06) ab	0.76 (0.05) a	1.37 (0.09) ab
60 mM NaCl	3.02 (0.16) a	1.37 (0.07) ab	0.98 (0.07) ab	1.47 (0.09) b
CT water	2.17 (0.16) a	1.49 (0.10) b	0.88 (0.07) ab	1.23 (0.08) ab

Table A26. ANOVA for shoot fresh weight over a 10-week treatment period of seedlings from 3 seed sources (SS) exposed to 6 salt pre-treatments (PT) at one-month-of-age, and 4 salt treatments (TRT) at 7-months-of-age, using 4 blocks of treatment trays (REP).

Source	df	Mean square	F	p value
SS	2	14.515	16.238	< 0.001
PT	5	7.417	8.297	< 0.001
TRT	3	319.916	357.893	< 0.001
REP	3	13.048	14.597	< 0.001
SS x PT	10	1.431	1.601	0.101
SS x TRT	6	1.622	1.814	0.093
PT x TRT	15	4.758	5.323	< 0.000
SS x PT x TRT	30	0.867	0.969	0.514
Error	1422	0.894		

Table A27. Mean (± SE) shoot fresh weight (g) over a 10-week treatment period of three seed sources. Combined data from all pre-treatments and treatments. Different letters represent significant differences between seed sources.

Seed source	Mean (g)
Syncrude	3.243 (0.057) a
Suncor	3.219 (0.053) a
Smoky Lake	3.547 (0.063) b

Table A28. Mean (± SE) stem elongation (cm) over a 10-week treatment period of seedlings treated for 10 weeks with CT water or salt solution. Combined data from seed sources. Different letters represent significant differences between pre-treatments within a treatment group.

	Treatment			
Pre-treatment	Control	60 mM NaCl	60 mM Na_2SO_4	CT water
Control	4.77 (0.14) ab	2.92 (0.12) a	3.13 (0.08) a	3.83 (0.12) a
30 mM Na_2SO_4	5.41 (0.15) b	3.08 (0.12) a	2.57 (0.09) ab	3.14 (0.11) ab
60 mM Na_2SO_4	5.09 (0.18) ab	2.98 (0.16) a	2.72 (0.14) ab	3.14 (0.19) ab
30 mM NaCl	5.00 (0.11) ab	3.30 (0.11) a	2.41 (0.09) ab	3.24 (0.12) ab
60 mM NaCl	5.06 (0.13) ab	3.20 (0.11) a	2.36 (0.08) b	3.28 (0.09) ab
CT water	4.42 (0.14) a	2.84 (0.10) a	2.61 (0.09) ab	2.78 (0.12) b

Field study (Chapter III)

Model: $Y_{ij} = \mu + \gamma_i + \delta_j + \varepsilon_{ij}$

Where γ_i represents pre-treatment (fixed factor) and δ_j represents block (random factor).

Linear regression (Chapters IV, V, VI, VII)

Model: $Y_{ij} = \mu + \beta (x_i - x) + \varepsilon_{ij}$

One-year-old seedlings treated for 5 weeks (Chapter VI)

Model: $Yijk = \mu + \gamma_i + \rho_j + (\gamma\rho)_{ij} + \delta_k + \varepsilon_{ijk}$

Where γ_i represents treatment (fixed factor), ρ_j represents time (fixed factor), $(\gamma\rho)_{ij}$ represents treatment by time interaction, and δ_k represents block (random factor).

Table A29. Concentrations of (mg kg^{-1} DW) Na, Cl, K, Ca, and Mg in roots of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Na	Cl	K	Ca	Mg
Controls	0	Mean	131	1275	6350	6098	1807
		S.E.	35	102	419	509	82
	1	Mean	254 a	1003 a	6192 a	6893 a	2127 a
		S.E.	23	94	573	313	35
	3	Mean	841 a	1327 a	8347 a	8250 ab	2717 a
		S.E.	118	168	1127	537	98
	5	Mean	777 a	1218 a	9748 a	12688 c	3321 a
		S.E.	113	193	1106	621	198
60 mM	1	Mean	2354 b	4967 c	5858 a	6280 a	1926 a
NaCl		S.E.	137	517	932	827	198
	3	Mean	8663 b	11230 c	3085 b	8265 ab	2308 b
		S.E.	947	1164	552	707	98
	5	Mean	10264 b	12058 c	2137 b	10452 b	2104 b
		S.E.	796	906	137	774	41
120 mM	1	Mean	3402 b	5553 c	4926 a	6696 a	1944 a
NaCl		S.E.	238	523	512	515	146
	3	Mean	11167 c	13074 c	2198 b	9897 b	2053 bc
		S.E.	1075	1253	198	1479	120
	5	Mean	13267 d	16764 d	>2000	9932 b	1720 b
		S.E.	708	2227		664	102
60 mM	1	Mean	3658 b	1246 a	4710 a	6410 a	2165 a
Na_2SO_4		S.E.	654	84	601	324	175
	3	Mean	10417 bc	1712 a	2357 b	7322 ab	1842 c
		S.E.	820	108	148	126	102
	5	Mean	11654 c	885 a	>2000	7964 a	1820 b
		S.E.	1077	123		620	126
60 mM	1	Mean	2926 b	3534 b	5096 a	7000 a	1986 a
NaCl +		S.E.	382	770	574	721	58
30 mM	3	Mean	11305 c	8026 b	2545 b	5742 a	2128 bc
Na_2SO_4		S.E.	1160	1001	320	332	49
	5	Mean	13170 d	7406 b	2277 b	7686 a	1741 b
		S.E.	1107	632	219	427	69

Table A30. Concentrations of (mg kg^{-1} DW) Cu, Fe, Zn, P, PO_4, S, and SO_4 in roots of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Cu	Fe	Zn	P	PO_4	S	SO_4
Controls	0	Mean	320	302	207	3850	7605	2687	5016
		S.E.	71	25	28	94	272	67	285
	1	Mean	305	451	169	4207	6602	2863 a	4919 a
		S.E.	56	34	36	139	392	102	312
	3	Mean	186	668	100	3878	5275	4343 b	4951 a
		S.E.	13	28	20	179	263	1041	560
	5	Mean	285	1005	159	6048 a	9587	3134 b	5194 b
		S.E.	27	101	24	575	1367	245	458
60 mM	1	Mean	362	389	212	4198	7913	2766 a	4699 a
NaCl		S.E.	95	38	48	447	1390	300	557
	3	Mean	405	612	254	3675	6214	2945 ab	5081 a
		S.E.	133	75	103	160	329	168	561
	5	Mean	457	825	244	3423 b	5165	2241 a	2612 a
		S.E.	166	53	101	230	485	174	455
120 mM	1	Mean	258	443	107	3920	6796	2678 a	4039 a
NaCl		S.E.	32	64	27	252	476	172	381
	3	Mean	292	687	110	3873	5566	2438 a	3278 a
		S.E.	68	50	33	246	615	185	683
	5	Mean	287	808	124	3200 b	4531	1920 a	1932 a
		S.E.	51	128	33	127	537	78	545
60 mM	1	Mean	283	403	196	3972	6440	4145 b	8867 b
Na_2SO_4		S.E.	47	22	38	334	352	311	1087
	3	Mean	155	529	79	3497	5016	7175 c	18350 c
		S.E.	19	24	18	189	515	329	1275
	5	Mean	339	747	184	2819 b	3825	7414 d	18807 d
		S.E.	49	47	33	194	544	424	1627
60 mM	1	Mean	568	427	393	4194	5662	3660 b	5891 a
NaCl +		S.E.	201	34	145	225	1363	345	1706
30 mM	3	Mean	257	609	133	3635	5825	4412 b	9288 b
Na_2SO_4		S.E.	83	58	57	103	385	221	539
	5	Mean	309	914	145	2937 b	4092	4911 c	10374 c
		S.E.	44	97	25	463	851	290	824

Table A31. Concentrations of (mg Kg^{-1} DW) Na, Cl, K, Ca, and Mg in stems of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Na	Cl	K	Ca	Mg
Controls	0	Mean	124	2920	3530	2806	1594
		S.E.	22	416	225	87	80
	1	Mean	189 a	2104	3753	3102 a	1642
		S.E.	41	362	179	202	101
	3	Mean	212 a	1489 a	5385 c	2670 bc	1445 b
		S.E.	57	272	323	188	95
	5	Mean	176 a	1121 a	6535 c	2683 bc	1522 b
		S.E.	41	109	504	162	94
60 mM NaCl	1	Mean	2518 b	1900	3308	3073 a	1698
		S.E.	294	348	287	191	163
	3	Mean	9900 bc	1701 a	3892 b	3184 c	1692 bc
		S.E.	899	85	404	294	163
	5	Mean	19306 c	1748 ab	3991 b	3530 c	2277 d
		S.E.	2342	219	349	307	168
120 mM NaCl	1	Mean	2800 b	1909	2945	3153 a	1895
		S.E.	263	362	357	120	108
	3	Mean	12557 c	2757 b	3780 b	3362 c	1908 c
		S.E.	1906	467	421	548	187
	5	Mean	14675 c	2592 bc	4622 b	3490 c	1953 cd
		S.E.	3282	275	962	491	157
60 mM Na_2SO_4	1	Mean	2105 b	2625	2968	2518 ab	1370
		S.E.	670	242	449	201	134
	3	Mean	6625 b	1297 a	2082 a	1883 a	775 a
		S.E.	859	319	82	91	49
	5	Mean	10253 b	3132 c	2446 a	1702 a	1010 a
		S.E.	1777	684	161	152	128
60 mM NaCl + 30 mM Na_2SO_4	1	Mean	2668 b	1643	3140	2438 b	1289
		S.E.	301	344	355	158	128
	3	Mean	11410 c	2207 ab	2787 ab	2400 ab	1630 bc
		S.E.	1010	412	312	124	92
	5	Mean	18714 c	2823 c	3278 ab	2430 b	1674 bc
		S.E.	3139	424	493	251	142

Table A32. Concentrations of (mg kg^{-1} DW) of Cu, Fe, Mn, Zn, P, PO_4, S, and SO_4 in stems of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Missing values for SE mean indicate a single sample for that treatment and time. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Cu	Fe	Mn	Zn	P	PO_4	S	SO_4
Controls	0	Mean	162	129	38	183	2304	2920	2060	3883
		S.E.	23	7	13	21	102	416	88	1051
	1	Mean	211	105	22	208	2292	2104	2042	2631
		S.E.	54	9	3	47	133	362	136	370
	3	Mean	201	43 a	5	178	1572 a	1489 a	1475 a	2210 a
		S.E.	66	13	1	85	130	272	104	265
	5	Mean	343	23 a	11	198	1247 a	1121	1638 a	2242 a
		S.E.	83	5	3	43	89	109	225	199
60 mM NaCl	1	Mean	252	123	23	233	2182	1900	1877	2223
		S.E.	60	7	4	52	137	348	131	437
	3	Mean	445	101 b	11	260	1920 ab	1701 a	1802 a	2874 a
		S.E.	357	19	4	165	162	85	134	376
	5	Mean	247	59 bc	BDL	186	1887 b	1748	2287 a	3070 a
		S.E.	46	8		97	116	219	138	345
120 mM NaCl	1	Mean	221	134	22	227	2297	1909	2253	2728
		S.E.	49	14	5	42	89	362	119	352
	3	Mean	103	80 ab	30	114	2387 b	2757 b	2130 a	3252 ab
		S.E.	30	16	15	21	230	467	193	563
	5	Mean	366	45 ab	24	237	2222 bc	2592	2183 a	2589 a
		S.E.	78	12		58	105	275	124	192
60 mM Na_2SO_4	1	Mean	375	129	22	286	2282	2625	3093	4239
		S.E.	157	15	8	98	108	242	779	557
	3	Mean	412	85 ab	13	237	1943 ab	1297 a	3555 b	7314 b
		S.E.	225	16	2	82	145	319	326	996
	5	Mean	336	62 bc	4	248	2318 c	3132	5002 b	11758 b
		S.E.	130	10		80	197	684	828	2486
60 mM NaCl + 30 mM Na_2SO_4	1	Mean	176	133	21	193	1910	1643	2088	3076
		S.E.	22	14	5	18	79	344	81	434
	3	Mean	253	96 b	10	211	2087 ab	2207ab	3342 b	6699 b
		S.E.	120	15	1	82	79	412	221	831
	5	Mean	282	73 c	BDL	203	2282 c	2823	4984 b	9942 b
		S.E.	70	4		51	203	424	793	1855

BDL – below detection limits

Table A33. Concentrations of (mg kg^{-1} DW) Na, Cl, K, Ca, and Mg in living needles of the previous years growth of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Na	Cl	K	Ca	Mg
Controls	0	Mean	57	835	6512	5098	2742
		S.E.	9	145	504	354	135
	1	Mean	137 a	948 a	3172 a	4738 a	2493 a
		S.E.	28	294	123	268	116
	3	Mean	244 a	1168 a	3510	8565 a	3172 ab
		S.E.	93	181	277	725	257
	5	Mean	70 a	984 a	4488 ab	8582 b	2898
		S.E.	22	66	250	392	180
60 mM NaCl	1	Mean	1016 ab	4854 b	4148 ab	5440 a	2605 a
		S.E.	248	695	313	473	191
	3	Mean	7932 b	28997 b	3447	8347 a	3370 ab
		S.E.	506	3228	213	420	280
	5	Mean	17590 c	23301 b	5407 b	8573 b	3330
		S.E.	4848	1942	1652	1100	145
120 mM NaCl	1	Mean	2188 b	8382 c	4375 b	6663 b	3188 b
		S.E.	674	1661	368	556	242
	3	Mean	12717 c	36570 c	4622	7670 a	3995 b
		S.E.	1204	2577	406	501	405
	5	Mean	17633 c	38835 c	7603 c	7517 b	3540
		S.E.	2242	6242	266	303	661
60 mM Na_2SO_4	1	Mean	979 ab	767 a	5482 c	4935 a	2487 a
		S.E.	328	115	332	215	143
	3	Mean	5042 b	1123 a	4233	5070 b	2602 b
		S.E.	594	70	335	443	98
	5	Mean	7943 b	1387 a	3503 a	5384 a	2696
		S.E.	866	268	308	511	108
60 mM NaCl + 30 mM Na_2SO_4	1	Mean	1225 ab	4013 b	5083 bc	5113 a	2353 a
		S.E.	442	953	285	401	113
	3	Mean	14835 c	23948 b	3883	7682 a	3748 a
		S.E.	2877	2047	943	294	440
	5	Mean	21800	33010	2940	7750	3270
		S.E.	.	.	.	.	.

Table A34. Concentrations of (mg kg^{-1} DW) Cu, Fe, Mn, Zn, P, PO_4, S, and SO_4 in living needles from the previous years growth of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Missing values for SE mean indicate a single sample for that treatment and time. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Cu	Fe	Mn	Zn	P	PO_4	S	SO_4
Controls	0	Mean	197	172	284	212	2663	3851	2382	3010
		S.E.	56	14	19	35	106	263	74	300
	1	Mean	156	134	228	191	1920	2515	2015 a	2560 a
		S.E.	34	11	33	27	122	271	130	194
	3	Mean	245	167	386 a	236	1747 a	2369 a	2902 a	4951 ab
		S.E.	74	16	48	37	199	330	315	905
	5	Mean	306	133 a	271 c	245	1339 a	1758 a	2721	4645 a
		S.E.	86	8	19	48	40	93	173 a	609
60 mM	1	Mean	244	158	262	242	2342	3366	2083 a	2411 a
NaCl		S.E.	47	17	28	29	266	724	210	405
	3	Mean	110	142	153 b	174	2125ab	2757 a	2172 a	2767 a
		S.E.	37	11	17	30	141	414	225	725
	5	Mean	715	131 a	57 a	611	2293 b	2078 a	2230 a	1566 a
		S.E.	258	21	46	206	203	165	357	383
120 mM	1	Mean	153	161	285	182	2632	3592	2703 a	3560 a
NaCl		S.E.	25	14	33	31	186	506	171	415
	3	Mean	159	157	119 b	218	2988 c	4563 b	2638 a	3278 a
		S.E.	54	17	19	37	199	537	292	606
	5	Mean	455	80 b	35 a	324	3017 c	3482 b	1807 a	992 a
		S.E.	248	12	18	102	385	858	324	448
60 mM	1	Mean	232	142	257	220	2422	3204	3743 b	7443 b
Na_2SO_4		S.E.	75	15	50	53	248	513	532	1798
	3	Mean	210	134	182 b	229	2065 ab	2702 a	6467 b	15631 c
		S.E.	98	11	24	92	143	451	838	2485
	5	Mean	129	125 a	156 b	145	2154 b	2497 a	7860 b	18419b
		S.E.	36	9	24	22	95	182	545	2461
60 mM	1	Mean	311	140	247	278	2113	3010	2477 a	4560 ab
NaCl +		S.E.	55	13	12	31	83	199	212	1131
30 mM	3	Mean	199	148	102 b	233	2482 bc	2825 a	4957 b	8414 b
Na_2SO_4		S.E.	72	34	23	52	396	402	1029	2081
	5	Mean	54	80	113	61	2930	6990	5120	11262
		S.E.								

Table A35. Concentrations of (mg kg^{-1} DW) Na, Cl, K, Ca, and Mg in necrotic needles of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Missing values for SE mean indicate a single sample for that treatment and time. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Na	Cl	K	Ca	Mg
Controls	3	Mean	281	466	4910	7900	3530
		S.E.	.	.	.	.	.
	5	Mean	217	699	3860	8700	3210
		S.E.	.	.	.	.	.
60 mM	3	Mean	8012 ab	19689	2488	10220 a	2916
NaCl		S.E.	642	3777	189	513	176
	5	Mean	13453 b	32578 b	3341 a	8513	2920
		S.E.	1049	1881	326	483	107
120 mM	3	Mean	6572 a	25010	3418	9222 a	3556
NaCl		S.E.	1717	4510	401	561	441
	5	Mean	12300 b	28867 b	4600 b	7572	2573
		S.E.	496	3166	327	350	165
60 mM	3	Mean	4670	1184	4580	9950	4290
Na_2SO_4		S.E.	.	.	.	.	.
	5	Mean	8365 a	1311 a	3657 ab	8008	3318
		S.E.	1559	397	380	1328	383
60 mM	3	Mean	13100 b	26505	2993	7308 b	3168
NaCl +		S.E.	2457	5724	407	412	226
30 mM	5	Mean	15717 b	26796 b	3415 a	7383	2807
Na_2SO_4		S.E.	1533	2466	345	505	220

BDL – below detection limits

Table A36. Concentrations of (mg Kg^{-1} DW) Cu, Fe, Mn, Zn, P, PO_4, S, and SO_4 in necrotic needles of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Missing values for SE mean indicate a single sample for that treatment and time. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Cu	Fe	Mn	Zn	P	PO_4	S	SO_4
Controls	3	Mean	147	242	314	202	2290	1495	2770	1748
		S.E.								
	5	Mean	130	297	801	181	1760	2136	2550	3689
		S.E.								
60 mM	3	Mean	65	193	519	1003	1676 a	1876	2374 a	2777 a
NaCl		S.E.	23	20	70	877	90	463	307	1185
	5	Mean	159	141	271	186	2032	2835	2144 a	2326 a
		S.E.	62	15	25	47	151	405	225	504
120 mM	3	Mean	96	171	394	157	2260 b	2816	2896 a	3262 a
NaCl		S.E.	14	25	102	28	146	412	145	284
	5	Mean	98	140	244	107	1970	2714	1863 a	1301 a
		S.E.	37	13	28	16	73	485	104	118
60 mM	3	Mean	203	214	998	273	1930	2136	9950	25243
Na_2SO_4		S.E.								
	5	Mean	88	169	473	158	2255	3175	10045 b	24272 c
		S.E.	37	36	195	51	365	1291	455	4854
60 mM	3	Mean	176	189	454	224	1918 ab	2655	5330 b	13350
NaCl +										b
30 mM		S.E.	74	15	41	63	219	458	1203	5279
Na_2SO_4	5	Mean	204	137	283	222	2177	3204	4967 c	9094 b
		S.E.	56	9	29	55	165	551	538	1555

BDL – below detection limits

Table A37. Concentration of (mg kg^{-1} DW) Na, Cl, K, Ca, and Mg in living needles of the current years growth in jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Missing values for SE mean indicate a single sample for that treatment and time. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Na	Cl	K	Ca	Mg
Controls	3	Mean	165 a	871 a	6958 ab	2457 a	1357
		S.E.	48	136	479	261	74
	5	Mean	1044 a	1112 a	6765 a	2995 a	1525
		S.E.	959	94	643	285	101
60 mM	3	Mean	8945 c	19515 c	10050 b	4185 b	1455
NaCl		S.E.	1555	3786	1650	265	205
	5	Mean	23400 c	44660 b	9380 a	4353 b	1497
		S.E.	839	9176	1229	788	53
120 mM	3	Mean	7280	12039	9350	4600	2180
NaCl		S.E.	.	.	.	.	.
60 mM	3	Mean	3560 b	1039 a	4865 a	2000 a	1410
Na_2SO_4		S.E.	660	223	235	220	60
	5	Mean	6830 b	1204 a	3987 b	2293 a	1543
		S.E.	1059	161	459	317	84
60 mM	3	Mean	14450 d	13786 b	7700 ab	3390 b	1465
NaCl +		S.E.	3350	194	1900	860	215
30 mM	5	Mean	24500	62136	6540	2030	1060
Na_2SO_4		S.E.	.	.	.	.	.

BDL – below detection limits

Table A38. Concentrations of (mg kg^{-1} DW) of Cu, Fe, Mn, Zn, P, PO_4, S, and SO_4 in living needles from the current years growth of jack pine seedlings at the beginning of the treatment period (time 0) and after 1, 3, or 5 weeks of treatment with salt solution. Missing values for SE mean indicate a single sample for that treatment and time. Different letters represent significant ($p \leq 0.05$) differences between treatments at a given time.

Treatment	Time (weeks)		Cu	Fe	Mn	Zn	P	PO_4	S	SO_4
Controls	3	Mean	140	27	30	116	BDL	1880	2440	1741 a
		S.E.	30	7	8	15		404	139	275
	5	Mean	336		22	217	BDL	1423 a	1647	2333 a
		S.E.	154		8	90		247	126	415
60 mM NaCl	3	Mean	231	28	27	204	BDL	1874	3345	500
		S.E.	110		6	72		456	115	
	5	Mean	136	24	25	143	BDL	2913 b	3150	718 a
		S.E.	55		16	49		388	120	175
120 mM NaCl	3	Mean	231	29	63	245	BDL	4078	4990	971
		S.E.								
60 mM Na_2SO_4	3	Mean	217	88	45	191	BDL	1845	2635	8738 b
		S.E.	49	55	2	17		291	75	1359
	5	Mean	670	33	48	506	BDL	2104 b	2533	8673 b
		S.E.	267		18	166		599	263	1907
60 mM NaCl + 30 mM Na_2SO_4	5	Mean	325	49	53	249	BDL	1476	3660	2718 a
		S.E.	192	2	15	86		39	970	194

BDL – below detection limit

Table A39. ANOVA for needle electrolyte leakage (% of total electrolytes) of seedlings exposed to 5 salt treatments (TRT) for 0, 1, 3, or 5 weeks (TIME), with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
TRT	4	508	16.709	< 0.001
TIME	3	170	5.593	0.002
REP	2	73	2.389	0.102
TRT x TIME	7	201	6.602	< 0.001
Error	53	30		

Table A40. Mean (± SE) needle electrolyte leakage (% of total electrolytes) over a 5-week treatment period of seedlings treated with salt solutions. Different letters represent significant differences between treatments within a time.

	Time (weeks)		
Treatment	1	3	5
Control	11.39 (3.43) a	6.82 (2.29) a	5.67 (0.79)
60 mM NaCl	15.50 (7.20) a	34.82 (7.91) c	14.85
120 mM NaCl	27.16 (8.30) b	25.26 (8.07) bc	17.83 (4.12)
60 mM Na_2SO_4	17.49 (4.90) a	16.31 (4.43) ab	25.29
60 mM NaCl + 30 mM Na2SO4	14.49 (3.56) a	36.73 (15.89) c	12.95 (7.36)

Table A41. ANOVA for stem electrolyte leakage (% of total electrolytes) of seedlings exposed to 5 salt treatments (TRT) for 0, 1, 3, or 5 weeks (TIME), with treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
TRT	4	164.9	5.357	0.001
TIME	3	42.9	1.392	0.255
REP	2	33.3	1.083	0.346
TRT x TIME	7	64.4	2.091	0.059
Error	57	30.8		

One-year-old seedlings treated for 3 weeks (Chapter VII)

Transpiration over time

Model: $\rho_{1ij} - \rho_{21ij} = \mu + \gamma_i + \varepsilon_{ij}$

Where γ_i represents treatment (fixed factor), and $\rho_{1ij} - \rho_{21ij}$ are the daily transpiration totals of the j_{th} subject in the i_{th} treatment at days 1 - 21.

Ion content, ion flux

Model: $Y_{ij} = \mu + \gamma_i + \rho_j + \varepsilon_{ij}$

Where γ_i represents treatment (fixed factor), ρ_j represents block (random factor).

Growth and water relations parameters

Model: $Y_{ijk} = \mu + \gamma_i + \rho_j + (\gamma\rho)_{ij} + \delta_k + \varepsilon_{ijk}$

Where γ_i represents treatment (fixed factor), ρ_j represents time (fixed factor) $(\gamma\rho)_{ij}$ represents treatment by time interaction, and δ_k represents block (random factor).

Table A42. ANOVA for shoot water potential of seedlings exposed to 5 salt treatments (TRT) for 0, 1 or 3 weeks (TIME), with blocks of treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
TRT	4	1.247	2.649	0.038
TIME	2	0.265	0.563	0.571
REP	2	2.437	5.177	0.007
TRT x TIME	4	0.296	0.629	0.643
Error	99	0.471		

Table A43. ANOVA for root hydraulic conductivity ($m^3\ g^{-1}$ root FW s^{-1} MPa^{-1}) of seedlings exposed to 5 salt treatments (TRT) for 0, 1 or 3 weeks (TIME), with blocks of treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
TRT	4	1.082E-13	3.080	0.020
TIME	2	2.257E-13	6.426	0.003
REP	2	1.382E-13	3.935	0.023
TRT x TIME	4	6.35E-14	1.808	0.135
Error	84	3.513E-14		

Table A44. Mean (± SE) root hydraulic conductivity ($m^3\ g^{-1}$ root FW s^{-1} MPa^{-1}) after a 1-week or 3-week treatment period of seedlings treated with salt solutions. Different letters represent significant differences between treatments within a time.

	Time (weeks)	
Treatment	1	3
Control	2.7 (0.51)	5.3 (0.82)
60 mM NaCl	2.0 (0.51)	3.5 (1.10)
120 mM NaCl	2.8 (0.55)	3.1 (0.75)
60 mM Na_2SO_4	1.9 (0.62)	4.0 (0.69)
60 mM NaCl + 30 mM Na2SO4	1.9 (0.57)	2.0 (0.47)

Table A45. ANOVA for root respiration (mmol O_2 kg^{-1} min^{-1}) of seedlings exposed to 5 salt treatments (TRT) for 0, 1 or 3 weeks (TIME), with blocks of treatment trays replicated 3 times (REP).

Source	df	Mean square	F	p value
TRT	4	8.261E-06	1.675	0.163
TIME	2	1.73E-05	3.507	0.034
REP	2	2.202E-07	0.045	0.956
TRT x TIME	4	4.777E-06	0.969	0.429
Error	83	4.931E-06		